BIOLOGICAL INDUSTRIES

2009-2011 CATALOGUE

PRODUCTS FOR ANIMAL CELL CULTURE & MOLECULAR BIOLOGY

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Dear Customer,

The aim of our new catalogue is to introduce you to Biological Industries' cell culture related products, and manufacturing services.
Our main mission is to provide you with products and services to maintain your cell culture under optimal growth conditions.
Over 25 years experience of constant innovation together with regulatory and performance challenges enable us to continue making products that you may rely on and provide you with service you can trust. Please note that all our prices are very reasonable.
In this catalogue you will find hundreds of cell culture-related products and complementary services that should provide one-stop-shopping solutions for your cell culture needs.

Biological Industries' Team

INTRODUCTION

Brief history and facts

- Biological Industries (BI) was established in 1981 in Western Galilee in the north of Israel at Kibbutz Beit Haemek. Our products are distributed in 35 countries worldwide. Our company is the exclusive distributor in Israel for 14 foreign suppliers. BI employs 60 people. We speak Hebrew, English, Arabic, Russian, Spanish, German and Dutch. BI is privately owned by kibbutz members and more than half of the employees are shareholders. Some 10,000 peer-reviewed scientific articles cite the use of cell culture reagents produced by BI.
- In the late 1980's BI was the pioneer of Serum-Free Media (SFM) development.
- In the 1990's BI was a pioneer[1] in serum-free media (SFM) research for many of the standard cell lines, e.g., hybridoma, CHO, Vero, etc.
- In 2004 BI launched its first product in the Stem Cells Initiative Product Line: Foetal Bovine Serum (FBS) pre-screened with hESCs. The QC is performed by the Stem Cells Technology Center at the Technion – Israel Institute of Technology. In 2005, BI launched a proprietary mouse embryonic stem cell medium that has outperformed competing products in the market in cell viability assays. In 2006, BI entered into an R&D collaboration with the Technion to develop Nutristem™, to be launched in 2009. The proprietary formulation is based on the co-invention by BI and Technion scientists that enables significant reduction in the costly recombinant growth factors, such as FGF, which is the main ingredient in competitors' products.
- In 2006 we established a new state-of-the-art manufacturing facility which is ISO standard for ISO 13485:2003. Production facilities consist of ISO 5, 7, 8 clean rooms.
- In 2008, in order to further upgrade our data transparency and quality control, an Enterprise Resource Planning (ERP) system was set up and currently all departments have achieved operational efficiency.
- In 2009 we will launch our new Nutriline™ product line, designed for customers who require chemically defined animal components-free (ACF) media. As part of this project, we collaborated with customers in order to develop special formulations for their unique cell line needs. BI is engaged in providing further innovations and establishing collaborations in this important area.

(1) Fiorentini D, Kaspi L, Talmon R, Bennett G. Versatile serum-free media formulations. Am Biotechnol Lab 1990 Sep; 8(11):35-7

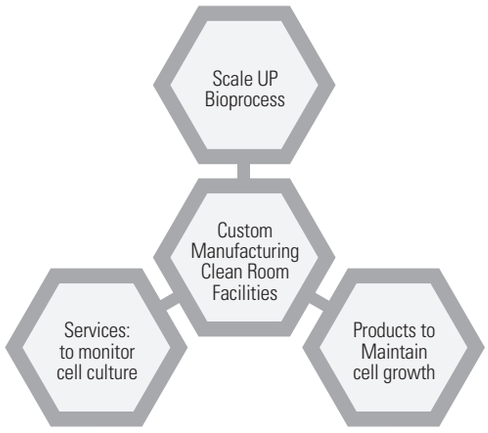
Over 25 years- Commitment to Provide Solutions for Maintaining Optimal Cell Culture Viability

BI manufactures and supplies to biopharmaceutical, academic and government research facilities, as well as to biopharmaceutical companies. Our diverse portfolio includes cell culture media, raw materials, auxiliary solutions and reagents, antibiotics, sera and bioprocess related products, services and tools. Animal component-based media is still the most common method to provide the nutritional need for growing cells. Therefore, the 1st chapter of our catalogue is dedicated to Feotal Bovine Sera (FBS) followed by the 2nd chapter- classical culture media that requires FBS supplementation. Our FBS is derived only from raw materials sourced from recognized EDQM-approved BSE/ FMD-free countries (Australia, New Zealand and Central America). It is filtered in a dedicated separate clean air system facility. This is our leading product that is sold globally to labs and industry. We are constantly looking for new industrial customers that can rely on our performance, quality and batch-to-batch reproducibility.

The 3rd chapter is dedicated to expansion of our contract manufacturing services in the fields of aseptic filling and cell culture-related services. One of the specific areas focuses on Animal Components Free (ACF) chemically defined media optimization, which is a key driver for market expansion, meeting bioprocess industrial needs:

- Elimination of animal products in research reagents that might contaminate future therapeutic products.
- Serum-free media provides the benefit of experimental control because there are fewer undefined components.
- Consistency in experiments that can be provided by quality controlled products.

BI's fully integrated approach for cell culture applications is based on the synergism between 25 years of accumulated expertise in cell culture and know-how, custom manufacturing service capabilities, a full range of cell culture related products and available customer support resources as shown in the figure below:



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The 4th chapter focuses on products for the stem cell scientist. The use of stem cells and other non-traditional cells for human therapy is becoming an area of increasing importance for media formulation. We are engaged in the development, production and sale of specialty research products for stem cell research.

The 5th chapter of this catalogue is devoted to our Serum-free (SF) Animal Component-Free (ACF) chemically defined media, which are required for predictability and consistency of cell culture bioprocess. Our ACF cryo-protecting cell freezing medium has shown superior performance in cell banks for industrial cell lines, such as HEK, CHO, etc., as well as for primary and stem cells. All products may be packaged in bulk per customer requirements.

Another major product line helps to easily monitor the viability of cell lines and prevent cell culture from mycoplasma contamination (Chapter 13), the major cell culture viability restricting factor. The proprietary PCR-based mycoplasma detection kit, which was co-developed with a leading academic institute, is ultra sensitive and rapid. The cell culture safe mycoplasma prevention and treatment product line may be used for cleaning water baths, incubators, work tools and surfaces. We provide a variety of standard antibiotic solutions and proprietary anti-mycoplasma solutions - BioMyc, to prevent high value-cell culture infection and to treat infected lines.

The major product line that accounts for a significant portion of the company's turnover is the portfolio for cytogenetic laboratories for prenatal diagnostic (e.g., amniocentesis), blood and bone marrow karyotyping, and related reagents (Chapter 11).

To summarize, most of our products target cell culture labs and provide high quality, cost-effective solutions that customers can rely on. Based on our accumulated culture media related know-how and aseptic filling expertise, we offer both products and customized manufacturing services for cell systems of both basic and applied research customers. The company regularly undergoes industrial partners' and ISO audits, thereby constantly adapting and upgrading its quality system to the rapidly changing regulatory environment. Utilizing our in-house expertise, we work with our clients to develop client-specific customized formulations to meet the unique needs of each cell culture client. We sell our products through a worldwide distributors' network (see Appendix) and have established a very strong presence in European and Asian countries alike. Most of our products bear the CE Mark and/or have EDQM approval (see Certifications in Appendix).

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TERM & CONDITIONS

1. ACCEPTANCE

1.a The terms and conditions of sale contained herein apply to all quotations made and purchase orders entered into by the Seller. Some of the terms set out here may differ form those in Buyer's purchase order and some may be new. This acceptance is conditional on Buyer's assent to the terms set out here in lieu of those in Buyer's purchase order. Seller's failure to object to provisions contained in any communication from Buyer shall not be deemed a waiver of the provisions of this acceptance. Any change in the terms contained herein must be specifically agreed to in writing by an officer of the Seller before becoming binding on either the Seller or the Buyer. These terms shall be applicable whether or not they are attached to or enclosed with the products to be sold or sold hereunder.

2. PAYMENT

2.a All invoices are due and payable thirty (30) days from the date of Air Waybill. No discounts are authorized. Shipments, Deliveries, and performance of work shall at all times be subject to the approval of the Seller and the Seller may at any time decline to make any shipments or deliveries or perform any work except upon receipt of payment or upon terms and conditions of security satisfactory to the Seller.

2.b If, in the Judgment of the Seller, the financial condition of buyer at any time does not justify continuation of production or shipment on the terms of payment originally specified, the Seller may require full or partial payment in advance and in the event of the bankruptcy or insolvency of the Buyer or in the event any proceeding is brought by or against the Buyer under; bankruptcy or insolvency laws, the Seller shall be entitled to cancel any order then outstanding and shall receive reimbursement for its cancellation charges.

2.c Each shipment shall be considered a separate and independent transaction, and payment therefore shall be made accordingly. If shipments are delayed by the Buyer, with consent of the Seller, payments shall become due on the date when the Seller is prepared to make shipment. If the work covered by the purchase order is delayed by the Buyer, with consent of the Seller, payments shall be made based on the purchase price and the percentage of completion. Products held for the buyer shall be at the risk and expense of the Buyer.

2.d Payment will be made in Israel according to the Seller's instructions. Without derogating from any right or remedy the Seller may have under law any amount not paid on time and in full shall bear interest from the date it became due and until the date of actual payment, at the maximum interest rate for the time prevailing at Bank Hapoalim in Israel.

3. TAXES

3.a The amount of any present or future sales, revenue, excise or other taxes, fees, or other charges of any nature, imposed by any public authority (national, state, local or other) applicable to the Products covered by the order, or the manufacturer or sale thereof, shall be added to the purchase price and shall be paid by the Buyer, or in lieu thereof, the Buyer shall provide the Seller with a tax exemption certificate acceptable to the taxing authority.

4. DELIVERY

4.a Where a period is named for delivery the Buyer shall take delivery within that period, unless otherwise expressly agreed by the parties in writing. The Buyer shall notify the Seller within 3 days of the arrival of the products at the port.

4.b Any time or date for delivery named by the Seller is an estimate only and is based upon prompt receipt from Buyer of all necessary information. In no event shall Seller be liable for re-procurement costs, nor for delay or non-delivery, due to causes beyond its reasonable control including, but not limited to, acts of civil or military authority, priorities, fires, strikes, lockouts, slowdowns, shortages, factory or labor conditions, errors in manufacture and inability due to causes beyond the Seller's reasonable control to obtain necessary labor, materials or manufacturing facilities. In the event of any such delay, the date of delivery shall at the request of the Seller, be deferred for a period equal to the time lost by reason of the delay.

4.c The Buyer at his own expense shall make provisions for the transport and/or collection of the Products from any port to which they may be sent by the Seller.

5. PASSING OF RISK AND PASSING OF TITLE

5.a From the date of arrival of the Products, as such date is specified in the Air Waybill, the risk of any loss or damage or deterioration of the Products due to whatever cause, shall be borne by the Buyer.

5.b The title in the Products shall remain vested in the Seller until the full and total consideration thereof shall have been paid to the Seller. Until the said full and total payment, the Buyer shall hold, at its expense, the Products on behalf of the Seller as bailee.

6. WARRANTY

6.a The Seller warrants that the products are free from defects in material and workmanship under normal use and service. Seller's obligations under this warranty are limited to replacing or giving credit for, at its option, at its factory, any of said Products which are after examination, disclosed to the Seller's satisfaction to be thus defective and which shall, at the Seller's option, be returned within

14 days after demand to the Seller's factory of origin, transportation charges repaid. The alleged defect, if any, shall not be a ground for cancellation of the remainder of the contract or order. This Warranty is expressed in lieu of all other warranties, expressed, statutory, or implied, including the implied warranties of merchantability and fitness for a particular purpose, and of all other obligations or liabilities on the Seller's part, and it neither assumes nor authorizes any other person to assume for the Seller any other liabilities in connection with the said Products or the sale thereof. This Warranty shall not apply to any Products which shall be subjected to misuse, negligence, or accident. Liability of the Seller, if any hereunder, shall in on event exceed in amount of the purchase price of the Products sold with respect to which claims are made. Seller neither assumes not authorizes any person to assume for it any other liability in connection with the sale or use of the Products sold hereunder, and there are no oral agreements or warranties collateral with or affecting this Agreement Seller shall not for any reason whatsoever be liable for loss of production or profits, or for other consequential losses which may be suffered or alleged to have been suffered by Buyer.

6.b The replacement or the giving of credit in accordance with the provisions of this paragraph above is subject to the procedure dictated by the Seller's insurance policy regarding the same and to the complete and accurate compliance by the Buyer with the instructions given by the Seller regarding the same.

7. CLAIMS

7.a No claims for damage in transit or loss of Products shall be entertained unless a complete claim in writing is given to the carrier concerned, and to the Seller and its agent (if any) within such time as will enable the compliance with the carrier's conditions of carriage as affecting damage in transit or loss of Products. Where Products are accepted from the carrier concerned without being checked, the delivery book of the carrier concerned must be signed "not examined".

8. DISTRIBUTORS

8.a For current information on Biological industries distributors and sales offices, please see distributors list in the appendix or use our website, www.bioind.com, or contact your local Biological Industries distributor to place your order. In the event that you do not have an authorized distributor, please contact:
Biological Industries
Kibbutz Beit-Haemek, Israel
Phone: 972-(0)4-9960595
Fax: 972-(0)4-9968896
E-mail: info@bioind.com

9. TECHNICAL SUPPORT

9.a Providing excellent technical support for our products is our top priority. Our technical service team relies on years of laboratory experience to assist you with product selection and advise to maximize product performance. You may reach our technical support via telephone/fax or email (see on the reverse side of the catalogue).

10. ONLINE ORDERING

10.a Online ordering for all of our products will be available on our newly constructed website www.bioind.com at the end of 2009. It will be accessible 24 hours/day, 7 days per week. Our online ordering system will be secure, fast, and convenient. Registered users will enjoy the ability to see their contracted prices, track orders, and manage their account.

11. GENERAL

11.a The sale of the Products by the Seller to the Buyer is subject to the length of the shelf-life of each Product as specified in the Seller's catalogue and, in the case of a specific order, according to such specific order.

11.b All disputes differences or questions at any time arising between the parties as to the construction, validity and performance of these terms and conditions of sale or to sales hereunder or as to any matter or thing arising there of or in any way connected therewith shall be referred to the arbitration of a single arbitrator in Israel. The arbitrator shall be appointed at the request of either party by the Chairman, of the time being, of the Manufacturers Association of Israel.

11.c If any such disputes, differences or questions not be submitted to arbitration and decided by an arbitrator as aforesaid, the exclusive jurisdiction to deal therewith shall be vested in the Courts of Tel Aviv, Israel

11.d These terms and conditions shall be subject to and construed in accordance with Israel law.

11.e If Buyer is in breach of its obligations under this contractor sale. Buyer shall remain liable for all unpaid charges and sums due to Seller and shall reimburse Seller for all damages suffered or incurred by Seller as a result of Buyer's breach. The remedies provided herein shall be in addition to all other legal means and remedies available to Seller under these terms and conditions of sale and under law.

NEW PRODUCTS

We are always searching for new, innovative products that help our customers maximize productivity and profitability. We offer different and superbly useful new products, some of which are mostly unique in characteristics.
Our new products for this 2009 catalogue are listed below.
You will find more detailed information regarding these new products in the specified chapters of the Catalogue:

04 Stem Cell Products

NutriStem™ hESC XF – Defined, Xeno-Free (XF) media, designed to support the growth of human embryonic stem cells (hESCs).

Product Name	Description	Catalogue No.	Page
NutriStem™ hESC XF medium With HSA	Xeno-Free, serum-free medium for hESCs	05-100-1	32
AF NutriStem™ hESC XF medium Without HSA	Xeno-Free, serum-free medium for hESCs	05-102-1	32
Bio-Pure HSA	Human Serum Albumin- optimized for human embryonic stem cells	05-720-1	35

05 Serum-Free and Animal Component-Free Media

Product Name	Description	Catalogue No.	Page
NutriVero VP1™	Animal component-free serum-free medium for monolayer culture of vero cells	05-066-1	42
NutriVero VP2™	Animal component-free serum-free medium for microcarrier suspension culture of vero cells	05-067-1	42
Cell Dissociation Solution (non-enzymatic)	Animal component-free Non-enzymatic cell dissociation solution	03-071-1	49
Papain Dissociation Solution	Animal component-free Cell dissociation solution	03-072-1	50
Accutase Solution	Accutase solution, primary human cell culture tested	03-073-1	51

07 Attachment Factors

Product Name	Description	Catalogue No.	Page
Gelatin Solution (0.1%)	0.1% gelatin solution qualified for mouse embryonic stem cells	0-944-1	63

11 Human Cytogenetics

Product Name	Description	Catalogue No.	Page
Cell Synchronization Kit	For high-resolution cytogenetic analysis	12-008-60	86

16 Molecular Biology

Product Name	Description	Catalogue No.	Page
RNA Save	Tissue storage solution for RNA stabilization	01-891-1	109
RNase-ExitusPlus™	Solution for the decontamination of RNase	01-895-1	109
DNA-ExitusPlus™	Solution for the removal of DNA & RNA contaminations	01-896-1	109

18 Human Serum and Blood Products

Product Name	Description	Catalogue No.	Page
EZ Lympho-Sep™	Ready-to-use lymphocyte separation tubes	01-899-U	125


BIOLOGICAL INDUSTRIES

SERA

Foetal Bovine Sera (FBS)
Bovine Sera
Other Sera

01

SERA

Introduction

Serum is commonly used as a supplement to basal growth medium in cell culture. The most common type of serum used for cell growth is foetal bovine serum (FBS), also known as foetal calf serum (FCS).
Foetal bovine serum is obtained from foetuses harvested in abattoirs from healthy dams fit for human consumption.
Occasionally, there may be use of other bovine sera, such as newborn calf serum or donor bovine serum. In cell culture, serum provides a wide variety of macromolecular proteins, low molecular weight nutrients, carrier proteins for water - insoluble components, and other compounds necessary for in vitro growth of cells, such as hormones and attachment factors. Serum also adds buffering capacity to the medium and binds or neutralizes toxic components. Attempts to replace serum entirely with serum-free medium have met only with limited success.
The selection of a serum supplement for cell culture applications is primarily dependent on the chemical definition of the basal medium, the type of cell to be grown, and the culture system being employed.

Collection
In the FBS manufacturing process, whole blood is collected aseptically in disposable sterile plastic bags and allowed to clot. Once the serum has been separated from the clot, it is pooled and frozen.
Controlling the initial collection of foetal blood is a crucial factor in the quality of the final serum product. Only raw material that meets our specifications is approved for production.

Handling Serum Products
Using Serum – Although the product has been sterile filtered, aseptic procedures must be followed at all times.

Granules, flocculent material or turbidity may develop after thawing. This particulate matter does not alter the performance of the serum as a supplement for cell culture medium. Repeated freezing/thawing of serum may increase the amount of precipitate and is therefore not recommended. If you do not intend to use an entire bottle of serum aliquot it into usable quantities in sterile containers before freezing a second time.

Wipe the outside of each bottle with a suitable disinfectant solution, before setting it on the work surface. Remove the heat seal and wipe the outside of the cap with the disinfectant solution.

All serum products should be treated as potentially harmful and appropriate care should be taken when handling them.

Raw Materials
Two distinct grades of FBS are available on the world market:
USDA-Grade FBS and European-Grade FBS.
USDA-Grade FBS is produced from raw materials originating only from countries certified to be free of both BSE (Bovine Spongiform Encephalopathy) and FMD (Foot and Mouth Disease). This product can be freely imported into any country, and is the product of choice in all countries for manufacturing purposes. Furthermore, only the use of this product allows researchers to send their cells, or the products of their cells, to collaborators in other countries with strict import regulations.
All FBS processed in the Biological Industries plant is USDA-Grade.

Processing
Selected batches of serum raw material are thawed, tested for endotoxins and hemoglobin content and only the accepted material is pooled. The pooled raw material is thoroughly blended under refrigerated conditions and membrane filtered for sterility according to a well validated filtration protocol. Biological Industries processes FBS through a sequence of pre-filters and membrane filters. The filtration step includes the use of three 0.1 micron sterilizing grade membrane filters in series.
After filtration, the serum is dispensed into bottles by an aseptic filling process which has been validated to insure sterility of the final product.
Serum products are produced in a controlled environment (clean rooms) designed to carefully control air pressure and particulate matter.
The manufacturing area is a class 100,000 (ISO 8) environment. The sterile bottles and equipment are stored in a class 10,000 (ISO 7) environment, and the filling room is a class 1000 (ISO 6) environment with class 100 (ISO 5) laminar air flow sterile bench.
Clean rooms are monitored on a regular basis for particulate and microbial levels to ensure that the air handling system, cleaning protocols and personnel maintain standards control.
After filling, the final product is quickly frozen to -20ºC and held in quarantine until all quality control tests have been completed.

Quality Control

Each lot of FBS is tested to confirm that the serum meets the written specifications. Final product release is done after reviewing all production and quality control records to determine compliance with all established, approved written procedures.

Physical and chemical tests
- Electrophoretic Pattern
- pH
- Osmolality

- Total proteins
- Albumin
- IgG
- Hemoglobin
- Globulins

Biochemicals Tests

The following tests are conducted on each lot of FBS:

- Alanine Transaminase (ALT)
- Alkaline Phosphatase
- Aspartate Aminotransferase (ast)
- Bilirubin – total
- Bilirubin – direct
- Blood Urea Nitrogen (BUN)
- Calcium
- Chloride
- Cholesterol
- Creatinine
- Creatinine Kinase (CK)
- Gamma-Glutamyl Transferase (GGT)
- Glucose
- High Density Lipoproteins (HDL)
- Lactate Dehydrogenase
- Low density Lipoproteins (LDL)
- Phosphorous (Inorganic)
- Potassium
- Sodium
- Triglycerides (TG)
- Uric Acid

Microbiological tests

- Sterility tests:
  Bacterial and fungal sterility tests according to the current USP
- Mycoplasma contamination:
  According to the Code of Federal Regulations (CFR), title 9, part 113 (culture method).
- Viral contaminants:
  According to the protocols described in CFR, title 9, part 113 for Bovine Viral Diarrhea (BVD), Infectious Bovine Rhinotracheitis (IBR) and Parainfluenza type 3 (PI3).
- Viral antibodies:
  FBS is screened to determine the titer of neutralizing antibodies to BVD, IBR and PI3.
- Endotoxins:
  The test is performed using the standard Limulus Amebocyte Lysate (LAL) with the kinetic turbidimetric method.

Biological performance (cell growth)

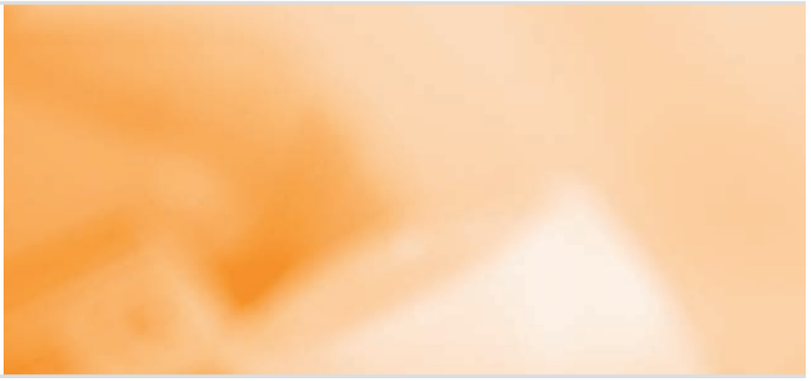
The cell growth tests are designed to check the efficacy of the FBS in promoting cell growth. Cells used are fibroblasts (MRC-5 diploid normal cells), epithelial cells Vero and hybridoma cells. Each test is conducted using the tested serum and a validated control lot. Growth promotion using MRC-5 cells is evaluated through several subculture generations to observe any evidence of cytotoxicity and morphological changes of the cells.
Vero cells (ATCC, CCL 81): plating efficiency.
MRC-5 cells (ATCC, CCL 171): 3 passages test.
Hybridoma cells: cell growth.

Stability

FBS stability at -20ºC temperature was evaluated with several cell types for long periods. The FBS did not lose its performance for 55 months (4.5 years) with all the cells tested. Storage of FBS at -20ºC without defrosting will maintain the quality of the FBS at least until the expiration date stated on the label.

Quality Assurance

The FBS production process is carried out under controlled conditions in a controlled environment. The steam-in-place (SIP) sterilization, filtration for sterility and filling are validated as required for key aseptic processes. A dossier (Device Master Record) exists for serum with all relevant data concerning serum production. The production process from the raw material to the final product in storage, as well as the quality control tests and results, are documented and filed to ensure traceability and control of the process.
Biological Industries' products are manufactured in compliance with the quality management standard ISO 9001:2000 and ISO 13485:2003. Certifications are available upon request.
In addition, the FBS production process conforms to the In Vitro Diagnostics Directive (IVDD 78/79/EC) of the European Parliament. Therefore, our FBS received the CE mark making it eligible for sale in the European Union for in vitro diagnostics.
A Bovine Spongiform Encephalopathy (BSE) Certificate of Suitability has been issued to Biological Industries by the European Directorate for the Quality of Medicines (EDQM) in accordance with monographs of the European Pharmacopeia.
All documents and certifications are available upon request.



Foetal Bovine Sera (FBS)

Serum, as a biological material, represents an undefined mixture of components in which composition varies from one lot to the other.
Some cell types are sensitive to the variations in serum performance. Customers are encouraged to evaluate serum samples with their own culture system and cells while we reserve the quantities of the specific lots until customer testing is completed. In this way, the customer may choose the best serum for his own applications.

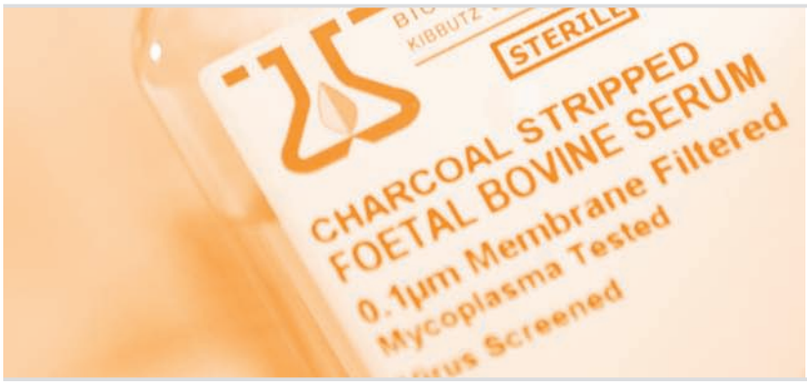
Biological Industries offers you the following certified sterile Foetal Bovine Serum Products:

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum	04-001-1A	500ml	-20ºC
	04-001-1B	100ml	-20ºC
Certified Foetal Bovine Serum Heat Inactivated	04-121-1A	500ml	-20ºC
	04-121-1B	100ml	-20ºC
Certified Foetal Bovine Serum Qualified for Human Embryonic Stem Cells	04-002-1A	500ml	-20ºC
	04-002-1B	100ml	-20ºC
Certified Foetal Bovine Serum Qualified for Human Embryonic Stem Cells Heat Inactivated	04-222-1A	500ml	-20ºC
	04-222-1B	100ml	-20ºC
Certified Foetal Bovine Serum Functionally Tested for use with Tetracycline Regulated Systems	04-005-1A	500ml	-20ºC
	04-005-1B	100ml	-20ºC
Certified Foetal Bovine Serum Functionally Tested for use with Tetracycline Regulated Systems Heat Inactivated	04-125-1A	500ml	-20ºC
	04-125-1B	100ml	-20ºC
Certified Foetal Bovine Serum Dialyzed	04-011-1A	500ml	-20ºC
	04-011-1B	100ml	-20ºC
Certified Foetal Bovine Serum Charcoal-Stripped	04-201-1A	500ml	-20ºC
	04-201-1B	100ml	-20ºC

Heat inactivated FBS

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum Heat Inactivated	04-121-1A	500ml	-20ºC
	04-121-1B	100ml	-20ºC

Heat inactivation of serum is performed by raising the temperature of the serum to 56ºC and maintaining that temperature for 30 minutes. Heat inactivation is the method of choice to destroy complement proteins activity.



FBS qualified for human Embryonic Stem (ES) Cells

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum Qualified for Human Embryonic Stem Cells	04-002-1A	500ml	-20°C
	04-002-1B	100ml	-20°C
Certified Foetal Bovine Serum Qualified for Human Embryonic Stem Cells Heat Inactivated	04-222-1A	500ml	-20°C
	04-222-1B	100ml	-20°C

Embryonic Stem (ES) Cells are pluripotent cells derived from the inner cell mass of the blastocyst. The stem cells can be maintained in vitro for extended periods without loss of their capacity to differentiate to all cell lineages when reimplanted back into a blastocyst. ES cells may differentiate in vitro to a variety of cell types including neuronal, muscle, endothelial and hematopoietic progenitors. General culture conditions are well established and usually require ES cells to be grown on an inactive feeder cell layer or with basement membrane components (Matrigel, Fibronectin, Laminin). When growing ES cells, one of the most important parameters is the maintenance of the cells in the undifferentiated state. Pre-screening of the serum is essential before using it for the culture of ES cells. Various lots of sera are screened for the growth of Human ES cells using MEF's as a feeder layer.

The following parameters are measured for the screening:

- Human ES cells colony morphology
- Plating efficiency
- Differentiation rate: analysis of Human ES cells surface marker expressed on the undifferentiated cells membrane (FACS analysis). The results are used as an indication of the quality of sera for the growth and maintenance of undifferentiated stem cells.

FBS - Functionally Tested for use with Tetracycline Regulated Systems

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum Functionally Tested for use with Tetracycline Regulated Systems	04-005-1A	500ml	-20°C
	04-005-1B	100ml	-20°C
Certified Foetal Bovine Serum Functionally Tested for use with Tetracycline Regulated Systems Heat Inactivated	04-125-1A	500ml	-20°C
	04-125-1B	100ml	-20°C

Functionally Tested for use with Tetracycline Regulated Systems FBS that have been pre-tested to ensure that they permit the range of tetracycline-regulated induction with well-characterized Tet lines.

Dialyzed FBS

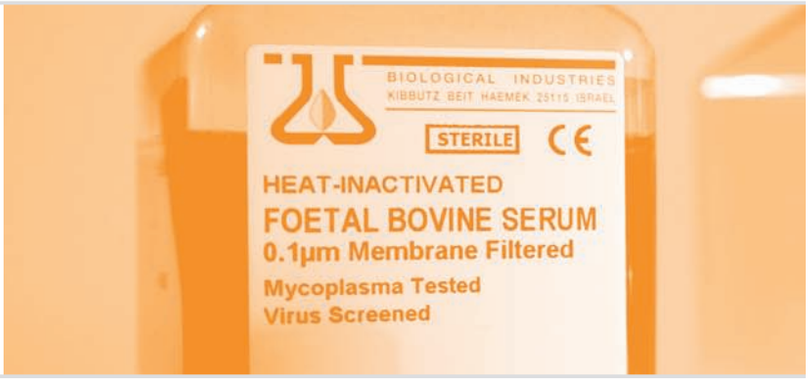
Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum Dialyzed	04-011-1A	500ml	-20°C
	04-011-1B	100ml	-20°C

Most cells grown in culture require a serum component of the growth medium to maintain their proliferative capacity. While whole serum is permissible for routine purposes, studies involving nutritional parameters or incorporation of labeled material require that the constituent under study be removed from the serum. The most commonly used method for removal of these constituents is dialysis of whole serum.
For dialysis by diafiltration, serum is circulated through a hollow-fiber by the concentration method. The filtrate, however, is replaced by the addition of physiological saline to the serum.

Charcoal-stripped FBS

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum Charcoal-Stripped	04-201-1A	500ml	-20°C
	04-201-1B	100ml	-20°C

Charcoal-stripped FBS is used to elucidate the effects of hormones in a variety of in vitro systems. Studies include steroid- receptor binding, steroid



regulation of cellular receptors, hormone secretion of various tissues and the function of thyroid hormones. The production procedure includes the use of charcoal and dextran to remove the hormones from the FBS.

Foetal Bovine Sera (FBS) FAQs

- **What is the difference between Foetal Bovine Serum (FBS) and Foetal Calf Serum (FCS)?**
  They are the same and describe exactly the same serum product.

- **My FBS contains precipitates. What are they and what should I do?**
  The precipitates contain fibrin (clot forming protein) and lipoproteins. This is a normal characteristic and will not affect product performance. To remove the precipitates, centrifuge the serum or simply let it settle to the bottom of the bottle and transfer the serum carefully to another sterile bottle.

- **My FBS arrived partially thawed, what should I do?**
  Let the serum defrost completely, swirl the serum bottle gently and then re-freeze the serum.
  The quality of the serum will not have been affected.

- **How do I heat-inactivate serum?**
  Serum heat-inactivation is performed in a waterbath at 56ºC for 30 minutes. The water level should be higher than the level of the serum. Monitor the temperature in a reference bottle containing water at the same volume during the heat inactivation. You must swirl the bottles to mix the serum every 5 minutes during heat inactivation to insure uniform heating. Use a calibrated thermometer only!

- **I have a jellylike fraction on the bottom of the bottle. What is it?**
  As a result of improper heat inactivation of the serum (temperature above 56ºC, more than 30 minutes), or inactivation without mixing the serum, a protein denaturation caused the jellylike fraction on the bottom of the bottle. Do not use this serum.

- **How should I thaw FBS?**
  We recommend thawing the serum at 2-8ºC. However, if necessary, you may thaw the serum at room temperature. Swirl the bottles gently to mix the serum during the thawing process.

- **Why is the color of the FBS not exactly the same as with my previous lot?**
  The color of FBS is brown to brown-red. It is dependent mainly on the hemoglobin concentration in the specific lot. The color does not affect the FBS performance.

- **Is the FBS raw material free of BSE?**
  FBS sold by Biological Industries has always been produced from raw materials originating from countries that are free of BSE according to the OIE (Office International des Epizooties).
- **How can I be certain regarding the country of origin of the raw material that was used to manufacture sterile FBS?**
  Ask Biological Industries for a copy of the original raw material veterinary documents.

Newborn Calf Sera

Product Name	Catalogue No.	Unit Size	Storage Temp.
Newborn Calf Serum (Less than 10 days)	04-102-1A	500ml	-20ºC
	04-102-1B	100ml	-20ºC
Newborn Calf Serum Heat Inactivated	04-122-1A	500ml	-20ºC
	04-122-1B	100ml	-20ºC

Bovine Sera

Product Name	Catalogue No.	Unit Size	Storage Temp.
Adult Bovine Serum	04-003-1A	500ml	-20ºC
	04-003-1B	100ml	-20ºC
Adult Bovine Serum Heat Inactivated	04-123-1A	500ml	-20ºC
	04-123-1B	100ml	-20ºC

Other Sera

Product Name	Catalogue No.	Unit Size	Storage Temp.
Donor Horse Serum	04-004-1A	500ml	-20ºC
	04-004-1B	100ml	-20ºC
Donor Horse Serum Heat Inactivated	04-124-1A	500ml	-20ºC
	04-124-1B	100ml	-20ºC
Porcine Serum	04-006-1A	500ml	-20ºC
	04-006-1B	100ml	-20ºC
Rabbit Serum	04-008-1A	500ml	-20ºC
	04-008-1B	100ml	-20ºC
Donor Goat Serum	04-009-1A	500ml	-20ºC
	04-009-1B	100ml	-20ºC


FOETAL BOVINE SERUM
0.1µm Membrane Filtered
Mycoplasma Tested
Virus Screened
GLYCOL-S

CLASSICAL CELL CULTURE MEDIA

Single Strength Liquid Media
Two Fold Concentration Media
Five & Ten Fold Concentration Media
Media For Insect Cells
Powdered Media

02

CLASSICAL CELL CULTURE MEDIA

Sterile Filtration and Aseptic Filling

Biological Industries' medium products are prepared by a sterile filtration process and aseptic filling. The process has been validated to ensure that the production of solutions meets the sterility assurance level of 10^3.
The filtration step includes the use of sterilizing grade membrane filters. After filtration, the medium is dispensed into bottles by an aseptic filling process which has been validated to insure sterility of the final product. Medium products are produced in a controlled environment (clean rooms) designed to carefully control air pressure and particulate matter.
The manufacturing area is a class 100,000 (ISO 8) environment. The sterile bottles and equipment are stored in a class 10,000 (ISO 7) environment, and the filling room is a class 1000 (ISO 6) environment with class 100 (ISO 5) laminar air flow sterile bench.
Clean rooms are monitored on a regular basis for particulate and microbial levels to ensure that the air handling system, cleaning protocols and personnel maintain required standards. After filling, the final product is held in quarantine until all quality control tests have been completed.

Quality Control

The quality of our liquid media is confirmed by testing representative samples from each lot.

Physicals Tests
pH & osmolality are measured to verify compliance with accepted specifications.

Endotoxins
Endotoxin concentrations are routinely measured with the Limulus Amebocyte Lysate (LAL) test using the kinetic turbidimetric method.

Sterility Testing
The absence of fungal and bacterial contamination is confirmed by sterility tests using the direct inoculation method or membrane filtration method with microbiological media. All media containing products of animal origin are tested for the absence of mycoplasma.

Cell Growth Promotion
The growth promotion activity and the absence of cytotoxicity of all medium products are tested using appropriate cell lines. Cells are examined for doubling time and cell morphology.

Expiration Date
Refer to product label for expiration date.

Storage
For optimal performance, store medium products under the conditions specified on the label.
Avoid light exposure of liquid medium products.

Certificate of Analysis and Safety Data
A Certificate of Analysis for each product lot is available upon request as well as a Material Safety Data Sheet (MSDS).

Single-Strength Liquid Media Products

Product Name	Catalogue No.	Unit Size	Storage Tamp.	Formulation Page
Basal Medium-Eagle (BME) **Earle's Salts Base** Without L-Glutamine	01-015-1A	500ml	2-8ºC	130 -131
	01-015-1B	100ml	2-8ºC	130 -131
Minimum Essential Medium-Eagle (MEM-E) **Earle's Salts Base** Without L-Glutamine	01-025-1A	500ml	2-8ºC	132
	01-025-1B	100ml	2-8ºC	132
Minimum Essential Medium-Eagle (MEM-H) **Hanks' Salts Base** Without L-Glutamine	01-035-1A	500ml	2-8ºC	132
	01-035-1B	100ml	2-8ºC	132
Minimum Essential Medium-Eagle (MEM-NEAA) **Earle's Salts Base** With Non-Essential Amino Acids Without L-Glutamine	01-040-1A	500ml	2-8ºC	132
	01-040-1B	100ml	2-8ºC	132
Minimum Essential Medium-Alpha (MEM-A) With 1g/l D-Glucose (Low Glucose) With L-Glutamine Without Ribonucleosides and Deoxyribonucleosides	01-042-1A	500ml	2-8ºC	131
	01-042-1B	100ml	2-8ºC	131

Product Name	Catalogue No.	Unit Size	Storage Tamp.	Formulation Page
Minimum Essential Medium Alpha (MEM-A) With 4.5 g/l D-Glucose (High Glucose) With L-Glutamine Without Ribonucleosides and Deoxyribonucleosides	01-043-1A	500ml	2-8ºC	
	01-043-1B	100ml	2-8ºC	
Minimum Essential Medium (MEM) for suspension cultures Without L-Glutamine	01-045-1A	500ml	2-8ºC	132
	01-045-1B	100ml	2-8ºC	132
Dulbecco's Modified Eagle Medium (DMEM) With 1g/l D-Glucose (Low Glucose) With Sodium Pyruvate 110mg/l Without L-Glutamine	01-050-1A	500ml	2-8ºC	130 -131
	01-050-1B	100ml	2-8ºC	130 -131
Dulbecco's Modified Eagle Medium (DMEM) With 4.5g/l D-Glucose (High Glucose) Without Sodium Pyruvate Without Phenol Red Without L-Glutamine	01-053-1A	500ml	2-8ºC	
	01-053-1B	100ml	2-8ºC	
Dulbecco's Modified Eagle Medium (DMEM) With 4.5g/l D-Glucose (High Glucose) Without Sodium Pyruvate Without L-Methionine Without L-Glutamine	01-054-1A	500ml	2-8ºC	
	01-054-1B	100ml	2-8ºC	
Dulbecco's Modified Eagle Medium (DMEM) With 4.5g/l D-Glucose (High Glucose) Without Sodium Pyruvate Without L-Glutamine	01-055-1A	500ml	2-8ºC	130 -131
	01-055-1B	100ml	2-8ºC	130 -131
Dulbecco's Modified Eagle Medium (DMEM) With 4.5g/l Glucose (High Glucose) Without Sodium Pyruvate With stable Glutamine	01-056-1A	500ml	2-8ºC	
	01-056-1B	100ml	2-8ºC	

Product Name	Catalogue No.	Unit Size	Storage Tamp.	Formulation Page
Dulbecco's Modified Eagle Medium (DMEM) Without D-Glucose Without Sodium Pyruvate Without L-Glutamine	01-057-1A	500ml	2-8ºC	
	01-057-1B	100ml	2-8ºC	
Iscove's Modified Dulbecco's Medium (IMDM) With L-Glutamine Without Alpha-Thioglycerol Without Beta Mercaptoethanol	01-058-1A	500ml	2-8ºC	130 -131
	01-058-1B	100ml	2-8ºC	130 -131
MCDB-153 (Modified)	01-059-1A	500ml	2-8ºC	
	01-059-1B	100ml	2-8ºC	
McCoy's 5A Medium (Modified) Without Serum With L-Glutamine	01-075-1A	500ml	2-8ºC	130
	01-075-1B	100ml	2-8ºC	130
Medium M-199 (M199E) Earle's Salts Base With L-Glutamine	01-080-1A	500ml	2-8ºC	131
	01-080-1B	100ml	2-8ºC	131
Medium M-199 (M199H) Hanks' Salts Base With L-Glutamine	01-085-1A	500ml	2-8ºC	131
	01-085-1B	100ml	2-8ºC	131
Nutrient Mixture F-10 (Ham's) With L-Glutamine	01-090-1A	500ml	2-8ºC	131
	01-090-1B	100ml	2-8ºC	131
Nutrient Mixture F-12 (Ham's) With L-Glutamine	01-095-1A	500ml	2-8ºC	131
	01-095-1B	100ml	2-8ºC	131
RPMI Medium 1640 With L-Glutamine	01-100-1A	500ml	2-8ºC	130
	01-100-1B	100ml	2-8ºC	130
RPMI Medium 1640 Without D-Glucose Without L-Glutamine	01-101-1A	500ml	2-8ºC	
	01-101-1B	100ml	2-8ºC	
RPMI Medium 1640 Without Phenol Red Without L-Glutamine	01-103-1A	500ml	2-8ºC	
	01-103-1B	100ml	2-8ºC	
RPMI Medium 1640 Without L-Glutamine	01-104-1A	500ml	2-8ºC	
	01-104-1B	100ml	2-8ºC	

Single-Strength Liquid Media Products (Cont.)

Product Name	Catalogue No.	Unit Size	Storage Tamp.	Formulation Page
RPMI Medium 1640 With 25mM Hepes With L-Glutamine	01-106-1A	500ml	2-8ºC	130
	01-106-1B	100ml	2-8ºC	130
Waymouth's MB 752/1 Medium With L-Glutamine	01-110-1A	500ml	2-8ºC	134
	01-110-1B	100ml	2-8ºC	134
Leibovitz L-15 Medium With L-Glutamine	01-115-1A	500ml	2-8ºC	132
	01-115-1B	100ml	2-8ºC	132
Dulbecco's Modified Eagle Medium (DMEM): Nutrient Mixture F-12 (Ham's) (1:1) Without L-Glutamine With Sodium Bicarbonate 1.2gm/l With Hepes 15Mm With Sodium Pyruvate 55mg/l	01-170-1A	500ml	2-8ºC	133
	01-170-1B	100ml	2-8ºC	133
Mouse Embryonic Stem Cells (ESC) Basal Medium With Stable Glutamine	01-171-1A	500ml	2-8ºC	
	01-171-1B	100ml	2-8ºC	

Two Fold Concentration Media

Product Name	Catalogue No.	Unit Size	Storage Tame.	Formulation Page
Minimum Essential Medium-Eagle (MEM-E) 2X Conc. Earle's Salts Base Without L-Glutamine With Sodium Bicarbonate	01-025-9A	500ml	2-8ºC	
	01-025-9B	100ml	2-8ºC	
Dulbecco Modified Eagle Medium (DMEM), 2X Conc. 4.5g/l D-Glucose (High Glucose) Without L-Glutamine With Sodium Bicarbonate	01-055-9A	500ml	2-8ºC	
	01-055-9B	100ml	2-8ºC	

Five-Fold and Ten-Fold Concentration Media

Product Name	Catalogue No.	Unit Size	Storage Tame.	Formulation Page
Basal Medium Eagle (BME) 10X Conc. Earle's Salts Base Without L-Glutamine Without Sodium Bicarbonate	01-015-5A	500ml	2-8ºC	128
	01-015-5B	100ml	2-8ºC	128
Minimum Essential Medium Eagle (MEM-E) 10X Conc. Earle's Salts Base Without L-Glutamine Without Sodium Bicarbonate	01-025-5A	500ml	2-8ºC	132
	01-025-5B	100ml	2-8ºC	132
Dulbecco Modified Eagle Medium (DMEM) 5X Conc. 1g/l D-Glucose (Low Glucose) Without L-Glutamine Without Sodium Bicarbonate	01-050-4A	500ml	AMB	128 -129
	01-050-4B	100ml	AMB	128 -129
Dulbecco Modified Eagle Medium (DMEM) 5X Conc. 4.5g/l D-Glucose (High Glucose) Without L-Glutamine Without Sodium Bicarbonate	01-055-4A	500ml	AMB	128 -129
	01-055-4B	100ml	AMB	128 -129
Medium M-199 10X Conc. Earle's Salts Base With L-Glutamine Without Sodium Bicarbonate	01-080-5A	500ml	2-8ºC	131
	01-080-5B	100ml	2-8ºC	131
Nutrient Mixture F-10 (Ham's) 10X Conc. With L-Glutamine Without Sodium Bicarbonate	01-090-5A	500ml	2-8ºC	
	01-090-5B	100ml	2-8ºC	
Nutrient Mixture F-12 (Ham's) 10X Conc. With L-Glutamine Without Sodium Bicarbonate	01-095-5A	500ml	2-8ºC	
	01-095-5B	100ml	2-8ºC	
RPMI Medium 1640 10X Conc. Without L-Glutamine Without Sodium Bicarbonate	01-104-5A	500ml	2-8ºC	130
	01-104-5B	100ml	2-8ºC	130

Media Preparation

Directions for the Preparation of Single Strength Synthetic Liquid Media (1x) from Concentrated Media.

1. Measure out sterile culture - grade water (Catalogue No. 03-055-1) to approximately 70% of desired total volume of media. Pour water into an appropriate sterile mixing container that is close to the desired final volume. The water should be at room temperature.
2. Add the amount of the concentrated medium or concentrated medium components.
3. Add the desired amount of L-Glutamine Solution 200 mM (Catalogue No. 03-020-1) if required.
4. Add the desired amount of Sodium Bicarbonate Solution 7.5% (Catalogue No. 03-040-1).
5. Add antibiotics solution if desired.
6. Add water to the final volume. During the dilution, stir gently into equilibrium. If necessary, adjust pH with sterile 1 N NaOH or HCl.
7. Add the desired amount of serum, if required.
8. Store at 2°C to 8°C.

Important

The above procedures are carried out under strict sterile conditions.
Do not use mouth pipetting.

Example 1

Preparation of Basal Medium-Eagle, Earle's Salt Base, one liter

1. 700 ml sterile water (Catalogue No.03-055-1)
2. 100 ml Basal Medium-Eagle, Earle's Salts Base, concentrate 10X, without Sodium Bicarbonate and L-Glutamine (Catalogue No. 01-015-5).
3. 10 ml L-Glutamine Solution 200 mM (Catalogue No. 03-020-1).
4. 29.4 ml Sodium Bicarbonate Solution 7.5%. (Catalogue No. 03-040-1).
5. 10ml Penicillin-Streptomycin Solution (Catalogue No. 03-031-1).
6. Sterile water to final volume, Adjust pH if necessary.

Example 2

Preparation of RPMI from Concentrate

Our RPMI Concentrate is prepared by a special method which enhances the stability of the product. Therefore in this case proceed as follows:

1. 700 ml distilled water
2. Add 100 ml RPMI Concentrate 10X
3. Adjust pH to 6.5-7.0 with 1N NaOH
4. Add 10.3 ml L-Glutamine Solution 200 mM
5. Add 26.7 ml Sodium Bicarbonate Solution 7.5%
6. Adjust pH with 1N NaOH or 1N HCl to pH 7.0-7.4
7. Add distilled water to final volume. Adjust pH if necessary.
8. Filter the medium into sterile containers using a 0.2 μm membrane filter.

Recommended Amounts of Sodium Bicarbonate and L-Glutamine To Be Added In The Preparation of Single Strength Liquid Media (1x) from Concentrated Media (5x, 10x)

Desired product Catalogue No. / Description	Prepared From product Catalogue No. / Description	Quantity **Sodium Bicarbonate** Solution 7.5% Catalogue No. 03-040-1 ml/Liter	Quantity **L-Glutamine** Solution 200mM Catalogue No. 03-020-1 ml/Liter
01-015-1 Basal Medium-Eagle Earle's Salts Base (1x)	01-015-5 Basal Medium-Eagle Earle's Salts Base (10x)	29.4	10
01-025-1 Minimum Essential Medium Eagle Earle's Salts Base (1x)	01-025-5 Minimum Essential Medium Eagle Earle's Salts Base (10x)	29.4	10
01-050-1 Dulbecco's Modified Eagle Medium Low Glucose (1x)	01-050-4 Dulbecco's Modified Eagle Medium Low Glucose (5x)	49.4	20
01-055-1 Dulbecco's Modified Eagle Medium High Glucose (1x)	01-055-4 Dulbecco's Modified Eagle Medium High Glucose (5x)	49.4	20
01-080-1 Medium M-199 Earle' Salt Base (1x)	01-080-5 Medium M-199 Earle' Salt Base (10x)	29.4	
01-085-1 Medium M-199 Hanks' Salt Base (1x)	01-085-5 Medium M-199 Hanks' Salt Base (10x)	4.7	
01-090-1 Nutrient Mixture F-10 (HAM) (1x)	01-090-5 Nutrient Mixture F-10 (HAM) (10x)	16	
01-095-1 Nutrient Mixture F-12 (HAM) (1x)	01-095-5 Nutrient Mixture F-12 (HAM) (10x)	15.7	
01-100-1 RPMI-1640 (1x)	01-104-5 RPMI-1640 (10x)	26.7	10.3
01-170-1 DMEM:F-12(1:1) (1x)	01-170-5 DMEM:F-12(1:1) (10x)	16	12.5

* Tryptose Phosphate Broth (2000mg/l) must also be added.
We recommend using cell culture grade water Cat. No. 03-055-1

L-Alanyl L-Glutamine Solution

Product Name	Catalogue No.	Unit Size	Storage Tamp.
L-Alanyl-L-Glutamine (Stable Glutamine) 200 mM in 0.85% NaCl	03-022-1B	100ml	-20ºC
	03-022-1C	20ml	-20ºC

L-Alanyl L-Glutamine is a dipeptide substitute for L-Glutamine.

- Can be used as a direct substitute for L-Glutamine at equimolar concentrations in mammalian cell culture systems.
- Eliminates problems associated with the spontaneous breakdown of L-Glutamine during incubation.
- Highly soluble in aqueous solution and is heat stable.

Expiration
24 months

Storage
-20ºC

Media for Insect Cells

Product Name	Catalogue No.	Unit Size	Storage Tamp.	Formulation Page
BIOINSECT-1 With Glutamine (ACFM)	05-050-1A	500ml	2-8ºC	
	05-050-1B	100ml	2-8ºC	
Schneider's Drosophila Medium With L-Glutamine	01-150-1A	500ml	2-8ºC	134
	01-150-1B	100ml	2-8ºC	134
Grace's Insect Cell Medium Without Insect Haemolymph Without Lactalbumin Hydrolysate Without Yeastolate With L-Glutamine	01-155-1A	500ml	2-8ºC	134
	01-155-1B	100ml	2-8ºC	134

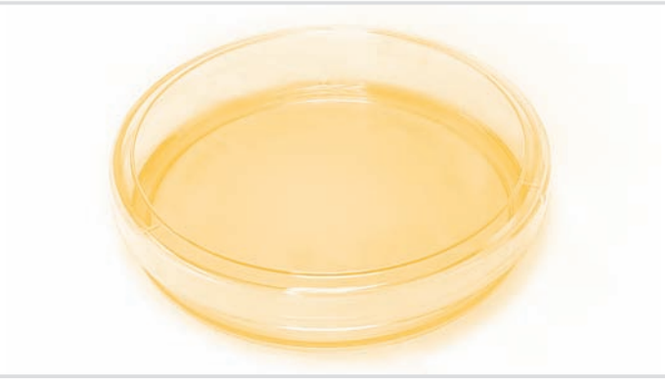
Powdered Media

Product	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Minimum Essential Medium-Eagle (MEM-E) Powder Earle's Salts Base With L-Glutamine Without Sodium Bicarbonate	11-025-1N	1x50 lt	2-8ºC	136-137
	11-025-1M	1x10 lt	2-8ºC	136-137
	11-025-1G	1x5 lt	2-8ºC	136-137
	11-025-1K	1x1 lt	2-8ºC	136-137
Minimum Essential Medium-Eagle (MEM-E) Powder Earle's Salts Base With Non-Essential Amino Acids With L-Glutamine Without Sodium Bicarbonate	11-040-1N	1x50 lt	2-8ºC	138-139
	11-040-1M	1x10 lt	2-8ºC	138-139
	11-040-1G	1x5 lt	2-8ºC	138-139
	11-040-1K	1x1 lt	2-8ºC	138-139
Minimum Essential Medium-Alpha (MEM-A) Powder With 1g/l D-Glucose (Low Glucose) Without Ribonucleosides and Deoxyribonucleosides With L-Glutamine Without Sodium Bicarbonate	11-042-1M	1x10 lt	2-8ºC	136-137
	11-042-1G	1x5 lt	2-8ºC	136-137
	11-042-1K	1x1lt	2-8ºC	136-137
Dulbecco's Modified Eagle Medium (DMEM) Powder With 1g/l D-Glucose (Low Glucose) With Sodium Pyruvate 110mg/l With L-Glutamine Without Sodium Bicarbonate	11-050-1N	1x50lt	2-8ºC	136-137
	11-050-1M	1x10lt	2-8ºC	136-137
	11-050-1G	1x5 lt	2-8ºC	136-137
	11-050-1K	1x1 lt	2-8ºC	136-137

Product	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Dulbecco's Modified Eagle Medium (DMEM) Powder With 4.5g/l D-Glucose (High Glucose) Without Sodium Pyruvate With L-Glutamine Without Sodium Bicarbonate	11-055-1N	1x10 lt	2-8ºC	136-137
	11-055-1M	1x10 lt	2-8ºC	136-137
	11-055-1G	1x5 lt	2-8ºC	136-137
	11-055-1K	1x1 lt	2-8ºC	136-137
Iscove's Modified Dulbecco Medium (IMDM) Powder With Hepes With L-Glutamine Without Sodium Bicarbonate	11-058-1N	1x50 lt	2-8ºC	138-139
	11-058-1M	1x10 lt	2-8ºC	138-139
	11-058-1G	1x5 lt	2-8ºC	138-139
McCoy's 5A Medium (Modified) Powder With L-Glutamine Without Sodium Bicarbonate	11-075-1M	1x10 lt	2-8ºC	138-139
	11-075-1G	1x5 lt	2-8ºC	138-139
Medium M-199 (M199E) Powder Earle's Salts Base With L-Glutamine Without Sodium Bicarbonate	11-080-1M	1x10 lt	2-8ºC	137-138
	11-080-1G	1x5 lt	2-8ºC	137-138
	11-080-1K	1x1 lt	2-8ºC	137-138
Nutrient Mixture F-10 (Ham's) Powder With L-Glutamine Without Sodium Bicarbonate	11-090-1M	1x10 lt	2-8ºC	137-138
	11-090-1G	1x5 lt	2-8ºC	137-138
	11-090-1K	1x1 lt	2-8ºC	137-138
Nutrient Mixture F-12 (Ham's) Powder With L-Glutamine Without Sodium Bicarbonate	11-095-1M	1x10 lt	2-8ºC	137-138
	11-095-1G	1x5 lt	2-8ºC	137-138
	11-095-1K	1x1 lt	2-8ºC	137-138
RPMI Medium 1640, Powder With L-Glutamine Without Sodium Bicarbonate	11-100-1N	1x50 lt	2-8ºC	138-139
	11-100-1M	1x10 lt	2-8ºC	138-139
	11-100-1G	1x5 lt	2-8ºC	138-139
	11-100-1K	1x1 lt	2-8ºC	138-139

Product	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Dulbecco's Modified Eagle Medium (DMEM): Nutrient Mixture F-12 (Ham's) (1:1), Powder With Hepes 15Mm With L-Glutamine Without Sodium Bicarbonate	11-170-1N	1x50 lt	2-8ºC	137-138
	11-170-1M	1x10 lt	2-8ºC	137-138
	11-170-1G	1x5 lt	2-8ºC	137-138
	11-170-1K	1x1 lt	2-8ºC	137-138
Dulbecco's Phosphate Buffered Saline (DPBS), Powder Without Calcium Chloride Without Magnesium Chloride With L-Glutamine Without Sodium Bicarbonate	11-223-1M	1x10 lt	2-8ºC	
	11-223-1G	1x5 lt	2-8ºC	
	11-223-1K	1x1 lt	2-8ºC	

Unit Sizes: 1 liter, 5 liters, 10 liters and 50 liters.
Other products and package sizes are available by special order.



Powdered Media Preparation Procedure:

1. To a mixing container that is as close to the final volume as possible, add 10% less distilled water than the desired total volume of medium.
2. Add powdered medium to room temperature water with gentle stirring. Do not heat water.
3. Rinse inside of package to remove all trace of powder.
4. Add Sodium Bicarbonate as required.
5. Dilute the medium to the desired volume with distilled water and stir until dissolved. Do not overmix.
6. Adjust the pH to between 0.2-0.3 below the desired final working pH by slowly adding, with stirring, 1N NaOH HCl. The pH usually will rise 0.2 -0.3 units upon filtration. Keep the container closed until the medium is filtered.
7. Process the medium immediately into sterile containers by membrane filtration using 0.2μ membrane filter.

See L-Alanyl L-Glutamine Solution on previous page.

Sodium Bicarbonate Concentrations:

Catalogue No.	Sodium Bicarbonate gram/liter	Sodium Bicarbonate ml/liter from 7.5% Solution
11-025-1	2.2	29.3
11-040-1	2.2	29.3
11-042-1	2.2	29.3
11-050-1	3.7	49.3
11-055-1	3.7	49.3
11-058-1	3.024	40.32
11-075-1	2.2	29.3
11-080-1	2.2	29.3
11-090-1	1.2	16
11-095-1	1.176	15.68
11-100-1	2.0	26.67
11-170-1	1.2	16

Example

RPMI 1640 (11-100-1) – 1 liter

1. Prepare 900ml of distilled water in clean glass beaker. Water temperature should be 15-30ºC. Put the beaker on a stirrer and add a stirring bar.
2. Add the 1-liter powder to the water and stir gently. Fill some distilled water into the empty package, stir and pour the remains into the beaker. Stir untill completely dissolved.
3. Add 2.0 gram Sodium Bicarbonate (or 26.67 ml of 7.5% Sodium Bicarbonate Solution).
4. Adjust pH to 0.1-0.3 units below the required pH using 1N HCl or 1N NaOH. The pH will rise by 0.1-0.3 units after filtration.
5. Add distilled water up to 1 liter.
6. Filter for sterility with 0.2μ membrane filter into sterile bottles.
7. For the preparation of 10 liters multiply by 10.

CUSTOM MANUFACTURING & LABORATORY SERVICES

03

CUSTOM MANUFACTURING & LABORATORY SERVICES

Cell culture media and production capacity are closely interdependent. Therefore, in addition to our ready-to-use standard cell culture related products we offer our clients custom manufacturing and media optimization services. We have entered into agreements with various industrial customers for cooperation in the optimization of specific formulations for specific cell lines and technologies. One specific area of ours is custom manufacturing service focusing on Animal Components - Free (ACF) chemically defined media optimization. Recently BI successfully completed a custom oriented project for the development of ACF media for Vero cells for a large industrial customer in the vaccine area. Currently, for the same customer, we are completing the development of specific auxiliary reagents as a continuation of this project. We believe that our accumulated know-how for a wide range of ACF and SFM products serves as a basis for starting collaboration and media optimization, and to scale up services for any cell lines requested by the customer.

Biological Industries (BI) specializes in the preparation of custom media according to the exact formulations and specifications for research in academia and industry. With more than twenty-five years of experience and an impressive reputation for quality, we can provide:

- Low volume to large scale production
- Rapid turn-around time
- Easy order/re-order process

All liquid media can be formulated from single-strength; two-fold and five to ten-fold concentrations to meet your special needs and requirements. Each lot undergoes strict and vigorous Quality Control Protocols to verify compliance with the product's specifications.

As a part of our fully integrated approach to cell culture related applications, our customer services have become a unique client-oriented application. We offer client-oriented confidential development, material transfer and optimization support for industrial customers.

Manufacturing Capabilities

- Liquid formulations: batch sizes from 6 to 1000 liters
- Customized packaging configurations- flexible packaging
- Consistency in small and large batches
- Expertise in SF and ACF media optimization per cell line
- Flexible product delivery
- Worldwide shipping and logistics capabilities

Manufacturing Facilities

- State-of-the-art clean room facilities.
- Animal origin materials from raw materials sourced only in BSE and FMD- free countries produced in a separate clean air facility.
- Autoclave, ultrafiltration, lyophilization, tissue culture, aseptic filling equipment
- Purified water (RO)

QA System Support

- Ongoing technical support
- Raw material supplier qualifications
  1. Elaboration of manufacturing and control SOPs
  2. Validated SOP for gamma irradiation
- Regular media fill
- BI is ISO 13485:2003 certified, a more specific supplement to ISO 9001, which includes medical device requirements relating to: design controls, process controls, special processes, traceability, record retention, and regulatory actions. Please see appendix for our regulatory certifications.

Laboratories Services (QC)

Test	Reference Standard
Sterility test	Current USP (71)
Biological Reactivity Test (cytotoxicity)- in-vitro (See chapter 15 – Cell Proliferation Kit)	Current USP (87)
Tests for cytotoxicity: in-vitro methods (See chapter 15 – Cell Proliferation Kit)	ISO 10993-5
Bacterial endotoxins (LAL reagent), kinetic turbidimetric method	Current USP (85)

Customized Media

Custom Formulations

Biological Industries specializes in the preparation of custom media according to the customer's exact formulations and specifications. This service is provided both to industrial and laboratory customers.
Our Custom-Formulated Media include:

- Balanced Salt Solutions
- Basal Media
- Complex Media
- Serum-Free Media
- Insect Cell Culture Media
- Plant Cell Media
- Powdered Media

The minimum quantity required for laboratories is only 12x500ml or 40x100ml. We can provide any media product in sterile bags of various sizes. We supply Sartorius-Stedim bags, but can fill any other type of bag provided by the customer.

Liquid Media

- Short supply time: 2-3 weeks
- Lots from 6 liters to 1000 liters
- Single strength (1x), Two fold (2x), Five fold (5x) and Ten fold (10x)
- Quality Controlled

Single-Strength Liquid Media

500ml size bottles Minimum order of 12 bottles Storage temperature 2-8ºC	or	100ml size bottles Minimum order of 40 bottles

Two-Fold Concentration Media

500ml size bottles Minimum order of 6 bottles Storage temperature 2-8ºC	or	100ml size bottles Minimum order of 30 bottles

Five & Ten Fold Concentration Media

500ml size bottles Minimum order of 6 bottles Storage temperature 2-8ºC	or	100ml size bottles Minimum order of 30 bottles

Powdered Media

- Supply time: 5-6 weeks
- Lots from 20 liters to 1000 liters
- Quality Controlled
- Package sizes available on request

We believe that the outsourcing of media optimization development and scale up is beneficial to bioprocess customers. Outsourced optimization services allow R&D customers to focus their technological resources on their core knowledge by eliminating the necessity to formulate media in-house and to invest in aseptic filling facilities and testing capabilities.

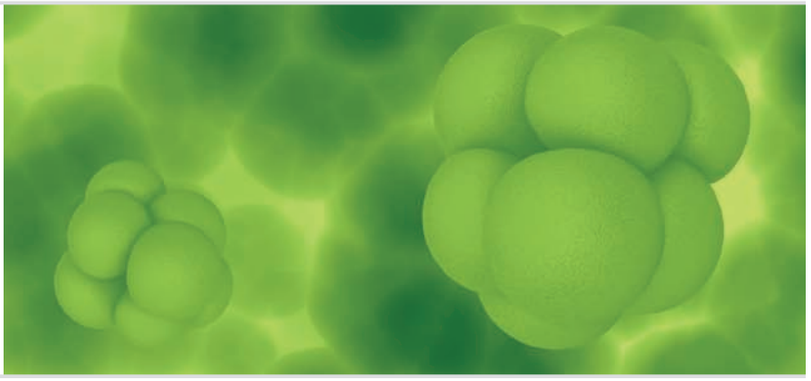
Price quotations for all of the above services are available upon request. For an immediate quote, simply contact Biological Industries locally at: 04-9960-496/595/596/597/686 or fax: 04-9960-631; 04-996-8896. Internationally at 972-4-9960-496/595, by fax: 972-4-9960631; 972-4-996-8896.

STEM CELL PRODUCTS

NutriStem™ - Human Embryonic Stem Cell products
Mouse Embryonic Stem Cell products
Foetal Bovine Serum- Qualified for Human Embryonic Stem Cells
Mesenchymal Stem Cell Media

04

STEM CELL PRODUCTS



The intricate relationship between genetics and embryonic development has both fundamental and practical implications for human health and disease. Stem cell research is probably one of the most exciting areas in the field of biology today due to the fact that it is advancing scientific knowledge exponentially in leaps and bounds, and raises as many questions as rapidly as it generates potential new discoveries. This promising area of scientific investigation provides a mechanism for creating animal-free component models with enormous therapeutic potential in Human Embryonic Stem Cell (hESC) research, and has also presented unforeseen and often startling insights into the role genes play in normal growth and development. Creating animal models of important human genetic diseases like cystic fibrosis (CF) or hemophilia is not only a prerequisite for developing and testing gene therapy, but embryonic stem cell technology also provides a testing ground to correct and repair genetic defects in utero.

Nutrients and other niche requirements for expanding the populations of therapeutic cells on a commercial scale are quite different from those needed for the production of cells in culture. An Animal Component-Free medium is a very important characteristic in the development of stem cells and regenerative medicine, as tissues are designed for implantation directly into humans[1]. Human embryonic and adult stem cells each have different characteristics regarding their potential use for cell-based regenerative therapies. The most obvious difference in the way embryonic and adult stem cells diverge is in the numbers and types of differentiated cell types they can become. In theory, embryonic stem cells, on the one hand, are pluripotent cells that have the ability to differentiate into all cell types of the body. Adult stem cells, on the other hand, are generally limited in terms of differentiation to the cell type tissue of origin. However, some recent evidence does suggest adult stem cell plasticity may exist, thus increasing the number of cell types an adult stem cell can become[2].

The utilization of Human Embryonic Stem Cells with its enormous therapeutic potential requires the development of serum-free and animal component-free media in order to close the gap between research models and clinical therapeutic procedures. This ever-increasing demand for media, free of any and all animal-derived components, is one of Biological Industries' top priorities.

[1] Genetic Engineering and Biotechnology News (GEN, Jan 15, 2009 pp 32-34). "Culture Media Underlies Productivity Gains," by Angelo DePalma,.

[2] The National Institutes of Health, U.S. Department of Health and Human Services, 2006.

Although serum supplementation has been, and probably will be, a crucial planning step that plays a vital and essential role in the success of your final medium in the near future, our Serum-Free (SF) and Animal Component-Free (ACF) medium has been successfully tested for Human Embryonic Stem Cell Culture. Each and every hESC batch is not only tested for its ability to maintain pluripotency and its differentiation capability, but also its normal morphology and karyotype in a serum-free environment.

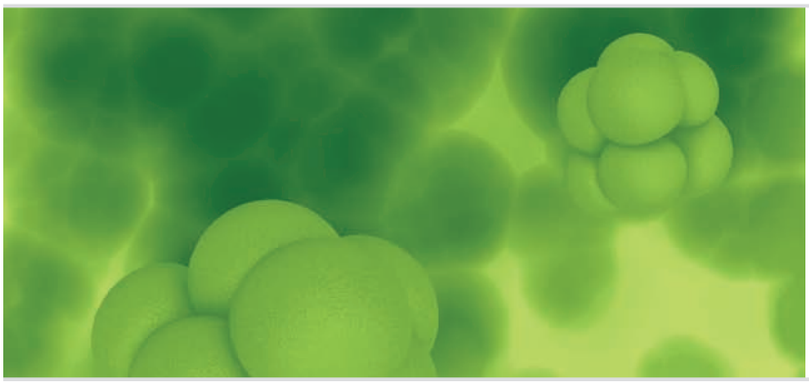
Until now, there were simply no real viable alternatives for a human embryonic stem cell culture without animal-derived components of any kind. Fortunately or unfortunately, serum or serum-like replacements were and still are necessary for the growth and proliferation of cells in culture. Although they have been characterized as an ill-defined "black box" mixture of all types of proteins, structural, carrier and functional proteins including growth factors, hormones, minerals, trace elements and even inhibitory substances, their inherently indefinable and wide variation of components with accompanied downtime for pre-screening and testing, have been all but eliminated with our fully defined, serum-free and animal component-free medium.

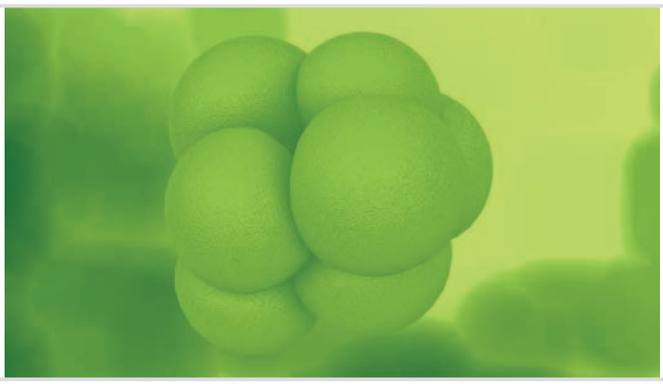
NutriStem™ hESC XF

Product Name	Catalogue No.	Unit Size	Storage Temp.
NutriStem™ hESC XF **Xeno-Free medium for hESCs** With HSA Optimized for FF culture, May be used with feeder-dependent culture Superior performance on Matrigel	05-100-1A	500ml	-20ºC
	05-100-1B	100ml	-20ºC
AF NutriStem™ hESC XF **Xeno-Free medium for hESCs** Without HSA Optimized for feeder-dependent culture	05-102-1A	500ml	-20ºC
	05-102-1B	100ml	-20ºC

Defined, xeno-free serum-free media, designed to support the growth of human embryonic stem cells (hESCs)

Traditional human Embryonic Stem Cells (hESC) culture methods require the use of mouse or human fibroblast feeder layers, or feeder-conditioned medium. These culture methods are labor-intensive, hard to scale up, and it is difficult to maintain hES cells undifferentiated due to undefined conditions. NutriStem™ hESC XF media were developed with a leading group in stem cell research, to enable the maintenance and expansion of hESCs with





feeder cells or on feeder-independent culture. NutriStem™ hESC XF support the culture of undifferentiated hESC in serum-free conditions without any animal components on mouse feeder cells (MEF), Matrigel or human foreskin fibroblasts (HFF). The media contain recombinant human basic fibroblast growth factor (rh bFGF) and recombinant human transforming growth factor (rh TGF). The media have been successfully tested and proven to maintain the pluripotential nature of hESCs.
For long-term growth of hESCs without feeder cells, the use of NutriStem™ hESC XF (with HSA) is recommended.

Features
Some predominant characteristics of our NutriStem™ hESC XF SF media include:
- A complete ready-to-use formulation (no additions are required). Contains Alanyl glutamine.
- Xeno-free: all components are defined and are of non-animal origin
- nables expansion of hESCs on feeder-free culture conditions (Matrigel) on human-feeder layer (foreskin fibroblasts) or on Mouse feeder cells (MEFs)
- High attachment – does not require adaptation period for feeder free culture
- Enables superior expansion of hESCs
- Supports long-term growth of hESCs (over 25 passages)
- Maintains differentiation capability of hESCs
- Maintains robust pluripotency
- Maintains normal phenotype (colony morphology) and genotype (karyotype testing) of hESCs
- Low proteins, low FGF levels
- Provides gene expression profiles comparable to classical media
- Intended for use in a 5% CO2 atmosphere (ordinary conditions)
- Consistent media performance
- Performance tested
- Sterile-filtered (0.1μ)
- Mycoplsma tested
- Endotoxin tested

Precaution and Disclaimer
1. For in vitro diagnostic and research use only.
2. Do not use if a visible precipitate is observed in the medium.
3. Do not use NutriStem™ hESC XF media beyond the expiration date indicated on the product label.

Storage and Stability
NutriStem™ hESC XF should be stored at -20ºC. Upon thawing, the media may be stored at 2-8ºC for 2 weeks. Dispense into aliquots to avoid repeated freezing and thawing.
Protect the media from light.

Quality Control
NutriStem™ hESC XF performance is tested for optimal maintenance and expansion of undifferentiated hESCs. Additional standard evalutions are pH, osmolality, endotoxins and sterility tests.

Figure 1a: hESCs (H9.2) growing on Matrigel (passage 5) express pluripotency markers as tested by Q-PCR. The results are expressed as percentage from GAPDH expression.

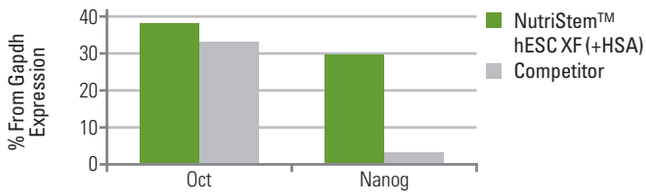


Figure 1b: hESCs (H9.2) growing on Human Foreskin Fibroblasts (HFF) (passage 5) express pluripotency markers as tested by Q-PCR. The results are expressed as percentage from GAPDH expression.

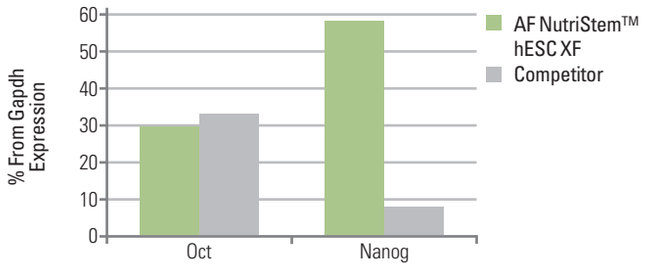


Figure 2: Evaluation of NutriStem™ hESC XF cultured human embryonic stem cells (H9.2 cells) using Matrigel as a matrix. Growth promotion for hESCs cultured in NutriStem™ hESC XF versus leading competitor. Cell counts are reported for days 2, 4 and 7.

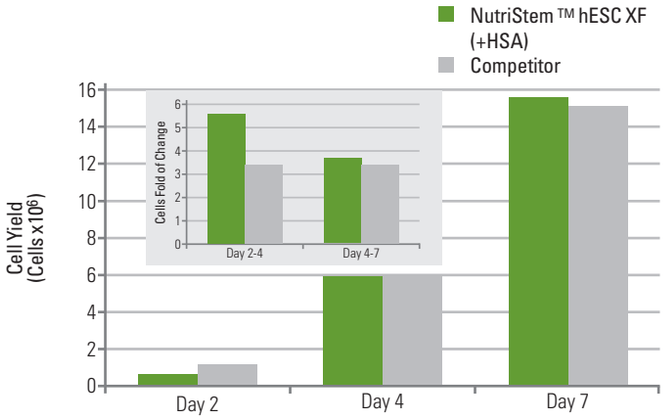


Figure 3: hESCs (H9.2) growing on Human Foreskin Fibroblasts (HFF) (passage 3) express pluripotency markers as tested by Q-PCR. The results are expressed as percentage from GAPDH expression.
The control is a basic medium and supplements routinely used in a leading stem cell laboratory.

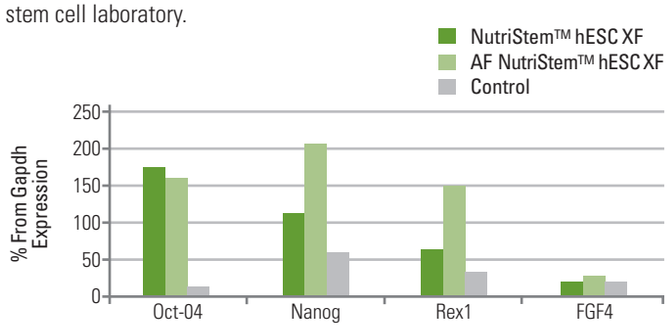


Figure 4: NutriStem™ hESC XF - Matrigel (>7 Passages).

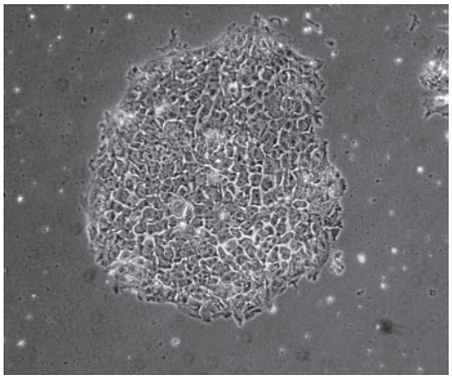
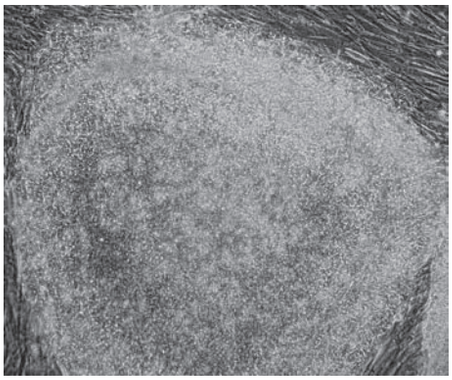
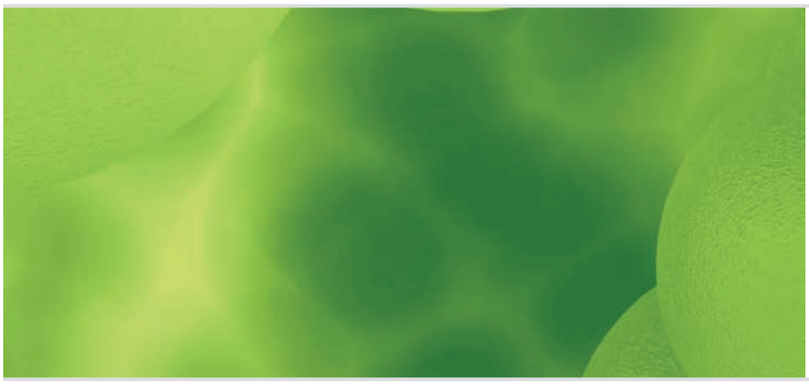


Figure 5: NutriStem™ hESC XF - HFF (>7 Passages).





Human Serum Albumin (HSA)

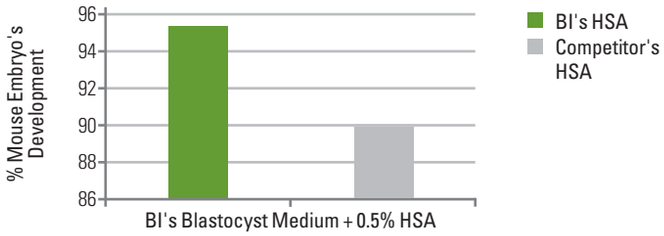
Product Name	Catalogue No.	Unit Size	Storage Temp.
Bio-Pure Human Serum Albumin (HSA Solution, 10%), Optimized for Human Embryonic Stem Cells (hESC)	05-720-1B	100ml	-20ºC
	05-720-1E	50ml	-20ºC

HSA is a medium supplement that is a highly soluble osmolytic protein with a high molecular weight. It was specifically developed to support and maintain cell development, growth, health and productivity in most cell culture media and especially cell membrane stability. The primary function of HSA is not only its unique demonstrative capability of binding anionic, cationic and neutral molecules, but it also has the proclivity of sequestering and stabilizing a wide array of ions and other small molecules.

HSA complies with the specifications of the manufacturer and the requirements stipulated by FDA approved tests.

All individual donations of the plasma and the corresponding plasma pool, has been tested for Hepatitis B Surface Antigen (HBsAg), Anti (Human Immunodeficiency Virus) HIV-I and II and anti-HCV and found to be negative.

Figure 6: 96-hour one-cell mouse embryo development in blastocyst medium supplemented with Biological Industries' 10% HSA solution (Cat. No.:05-720-1) versus competitor's 10% HSA solution.



Mouse Embryonic Stem Cells Basal Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
Mouse Embryonic Stem Cells (ESC) Basal Medium With L-Alanyl L-Glutamine	01-171-1A	500ml	2-8ºC
	01-171-1B	100ml	2-8ºC

Basal medium designed for the growth of mouse embryonic stem (ES) cells

Mouse embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the blastocyst. Undifferentiated ES cells can be maintained in-vitro for extended periods without loss of their capacity to differentiate to all cell lineages when reimplanted back into a blastocyst. ES cells may differentiate in-vitro to a variety of cell types including neuronal, muscle, endothelial and hematopoietic progenitors. General culture conditions are well established and usually require ES cells to be grown on an inactive feeder cell layer or on gelatin-coated plates with Leukemia Inhibitory Factor (LIF) in the culture medium.
Mouse ES Basal Medium has been optimized to grow and maintain undifferentiated mouse embryonic stem cells. The medium may be used with the addition of Foetal Bovine Serum (FBS) or with any serum replacement designed for mouse ES cells.
The medium contains L-glutamine in a stable form.

Storage
Mouse ES Basal Medium should be kept at 2-8ºC. Protect the medium from light.

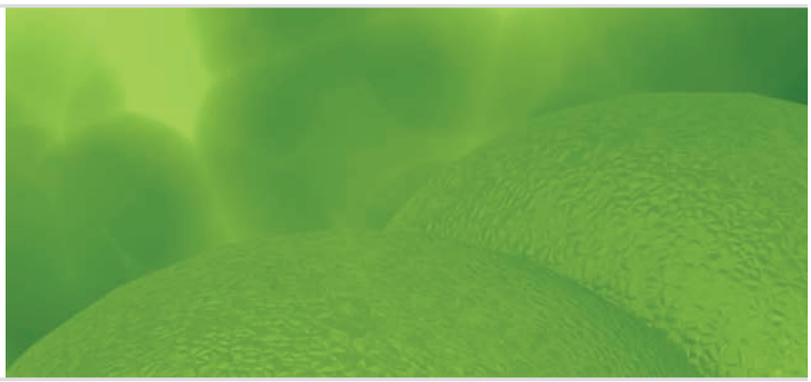
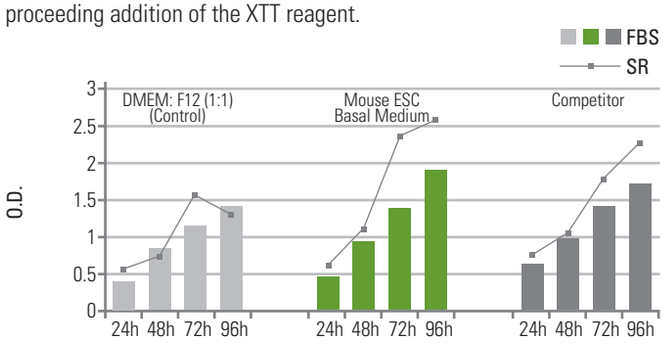


Figure 7: Growth rate of mouse ESC (ES-D3) using Biological Industries' Mouse ESC Basal Medium vs. a competitor's medium.
mESC were cultivated in a 96 well plates and observed over a period of 5 days. Proliferation rate was measured using a colorimetric method (XTT based Cell Proliferation Kit , Cat. No. 20-300-1000). Absorbance was read after 4 hours of incubation (wavelength of 450nm and reference of 690nm), proceeding addition of the XTT reagent.



Gelatin Solution, 0.1%

Product Name	Catalogue No.	Unit Size	Storage Temp.
Gelatin Solution (0.1%)	01-944-1A	500ml	2-8ºC
	01-944-1B	100ml	2-8ºC

Qualified for Mouse Embryonic Stem (ES) Cells

Gelatin solution (0.1%) is intended for coating cell culture flasks or plates used for the growth of mouse ES cells without feeder layer and with the addition of LIF to the culture medium.

Foetal Bovine Serum-Qualified for Human Embryonic Stem (ES) Cells

Product Name	Catalogue No.	Unit Size	Storage Temp.
Certified Foetal Bovine Serum (FBS) Qualified for Human Embryonic Stem Cells	04-002-1A	500ml	-20ºC
	04-002-1B	100ml	-20ºC
Certified Foetal Bovine Serum (FBS) Qualified for Human Embryonic Stem Cells Heat Inactivated	04-222-1A	500ml	-20ºC
	04-222-1B	100ml	-20ºC

Recommended Applications

For proliferation and differentiation of pluripotent embryonic stem cells. Based on high growth promotion parameters and lack of toxicity in highly sensitive human embryonic cell lines, this product is also recommended for sensitive mouse embryonic stem cells, human adult stem and primary cells.

In order to maintain highly viable stem cells in culture, high concentrations of growth factors, hormones and other growth stimulating additives are needed. To provide growth factors in constant concentrations, the FBS must be tested with embryonic stem cells for performance efficiency. Biological Industries ES cell-qualified FBS is of high quality and is produced only from raw materials originated in FMD and BSE free countries. Only pre-selected batches are performance tested and ES cell-qualified, after reliability and reproducibility are ensured for the cultivation of human embryonic stem cells.

Predominant Features

- Special pre-selected serum batches.
- For human embryonic stem cells (hESCs).
- For expansion and differentiation of stem cells.

Foetal Bovine Serum, tested on human embryonic stem cells, is specifically tested for the ability to sustain undifferentiated cellular morphology of embryonic stem cells. Only suitable batches are selected and kept for stem research clients. Screening FBS batches for Biological Industries is performed at the Technion Institute of Technology in the Human Embryonic Stem Cells Laboratory of Prof. Joseph Itskovitz-Eldor M.D., D.Sc.,Faculty of Medicine-Stem Cells Research Center in Israel. The group at the Technion has extensive experience in the derivation and maintenance of human ES cell lines, their sub-clones and in their in vitro differentiation procedures. To meet acceptance criteria, the cells are cultured with MEFs as feeder layer for at least four passages during which the following parameters are measured:

- Colony Morphology. The colony morphology of the undifferentiated cells is expected to remain similar to that of cells cultured with the control medium, namely round colonies with clear borders and determined by direct observation (see Figures 4&5).
- Plating Efficiency. The accepted cloning efficiency should be in the expected range which is normal for hESCs cultured with FBS and measured by counting surviving colonies (Amit et al., Dev Biol, 2000).
- Background Differentiation rates determined by morphology and determined by FACS analysis for SSEA4 for pluripotency markers.

Advantages

- Only pre-screened and only those FBS batches selected that can provide the different growth factors necessary for stem cells growth promotion
- Selected FBS batches tested for consistent quality to insure batch to batch consistency.
- Promote the formation of aggregates known as embryoid bodies, important intermediates for further differentiation into neuronal or hematopoietic progenitors.

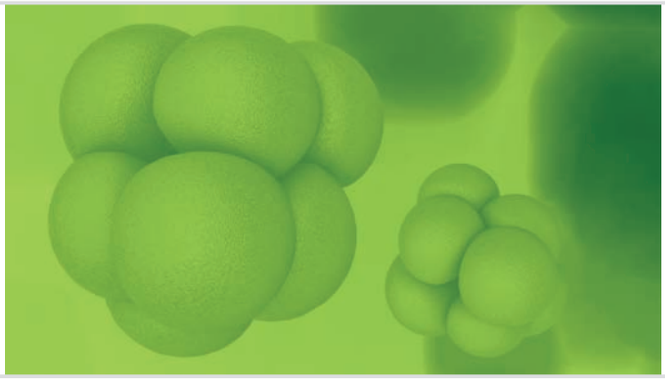
Mesenchymal Stem Cell Media

Product Name	Catalogue No.	Unit Size
Mesenchymal Stem Cell Growth Medium (Ready-to-use)	05-300-1A	500ml
Mesenchymal Stem Cell Adipogenic Differentiation Medium (Ready-to-use)	05-301-1B	100ml
Mesenchymal Stem Cell Chondrogenic Differentiation Medium (Ready-to-use)	05-302-1B	100ml
Mesenchymal Stem Cell Osteogenic Differentiation Medium (Ready-to-use)	05-303-1B	100ml

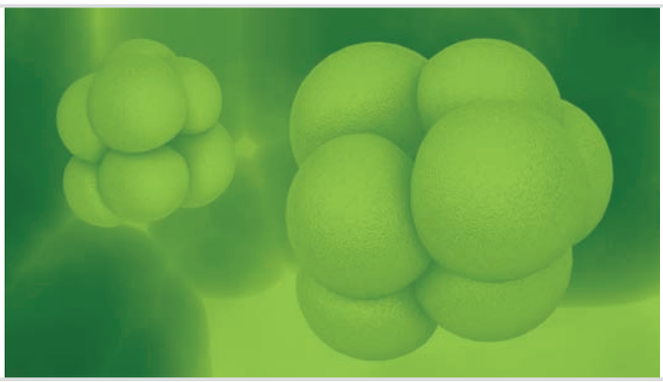
The Mesenchymal Stem Cell Growth Medium supports the expansion of multipotent human Mesenchymal Stem Cell (MSC) without inducing early senescence and differentiation as observed in standard culture media.
In addition, Biological Industries offers three media to efficiently induce differentiation of MSC into adipogenic, chondrogenic, or osteogenic lineages, respectively.

Recommended for:

- Human Mesenchymal Stem Cells from Bone Marrow (hMSC-BM).
- Human Mesenchymal Stem Cells from Umbilical Cord Matrix (hMSC-UC).
- Human Mesenchymal Stem Cells from Adipose Tissue (hMSC-AT).



Product Name	Catalogue No.	Unit Size	Storage Temp.
Human Embryonic Stem Cells products			
NutriStem™ hESC XF Xeno-Free Serum-Free medium for hESC With HSA	05-100-1A	500ml	-20ºC
	05-100-1B	100ml	-20ºC
AF NutriStem™ hESC XF Xeno-Free Serum-Free medium for hESC Without HSA	05-102-1A	500ml	-20ºC
	05-102-1B	100ml	-20ºC
Bio-Pure Human Serum Albumin (HSA Solution, 10%), Optimized for Human Embryonic Stem Cells (hESC)	05-720-1B	100ml	-20ºC
	05-720-1E	50ml	-20ºC
Mouse Embryonic Stem Cells products			
Mouse Embryonic Stem Cells (ESC) Basal Medium, with L-Alanyl L-Glutamine	01-171-1A	500ml	2-8ºC
	01-171-1B	100ml	2-8ºC
Gelatin Solution (0.1%)	01-944-1A	500ml	2-8ºC
	01-944-1B	100ml	2-8ºC
Foetal Bovine Serum Qualified for hESC			
Certified Foetal Bovine Serum (FBS) Qualified for Human Embryonic Stem Cells	04-002-1A	500ml	-20ºC
	04-002-1B	100ml	-20ºC
Certified Foetal Bovine Serum (FBS) Qualified for Human Embryonic Stem Cells Heat Inactivated	04-222-1A	500ml	-20ºC
	04-222-1B	100ml	-20ºC
Mesenchymal Stem Cell Media			
Mesenchymal Stem Cell Growth Medium (Ready-to-use)	05-300-1A	500ml	2-8ºC
Mesenchymal Stem Cell Adipogenic Differentiation Medium (Ready-to-use)	05-301-1B	100ml	2-8ºC
Mesenchymal Stem Cell Chondrogenic Differentiation Medium (Ready-to-use)	05-302-1B	100ml	2-8ºC
Mesenchymal Stem Cell Osteogenic Differentiation Medium (Ready-to-use)	05-303-1B	100ml	2-8ºC
Related Solutions			
Dulbecco's Modified Eagle Medium (DMEM): Nutrient Mixture F-12 (Ham's) (1:1) Without L-Glutamine With Sodium Bicarbonate 1.2gm/l With Hepes 15mM With Sodium Pyruvate 55mg/l	01-170-1A	500ml	2-8ºC
	01-170-1B	100ml	2-8ºC
MEM Non-Essential Amino Acids Solution, 100X Conc.	01-340-1B	100ml	2-8ºC
Human Recombinant Insulin Solution, 3.7 mg/ml	01-818-1H	5ml	2-8ºC
Serum-Free Cell Freezing Medium *	05-065-1A	500ml	2-8ºC
	05-065-1C	20ml	2-8ºC



Product Name	Catalogue No.	Unit Size	Storage Temp.
L-Glutamine Solution, 29.2mg/ml in Saline, 200 mM	03-020-1A	500ml	-20ºC
	03-020-1B	100ml	-20ºC
	03-020-1C	20ml	-20ºC
L-Alanyl-L-Glutamine (Stable Glutamine), 200 mM **	03-022-1B	100ml	-20ºC
	03-022-1C	20ml	-20ºC
Sodium Pyruvate Solution, 11.0mg/ml (100 mM)	03-042-1B	100ml	-20ºC
Crystalline Trypsin Solution (0.02%) Without Phenol Red *	03-047-1A	500ml	-20ºC
	03-047-1B	100ml	-20ºC
Soybean Trypsin Inhibitor 50X Conc., 5mg/ml *	03-048-1C	20ml	-20ºC
Cell Dissociation Solution (non-enzymatic) *	03-071-1B	100ml	2-8ºC
Papain Dissociation Solution *	03-072-1B	100ml	-20ºC
Fibronectin Solution (Bovine), 1mg/ml *	03-0901-01	1ml	2-8ºC
	03-0901-05	5ml	2-8ºC
Accutase Solution, primary human cell culture tested *	03-073-1B	100ml	-20ºC
Transferrin, Human, Substantially Iron-Free (APO) ***	41-951-100	100 mg	2-8ºC
	41-951-500	500 mg	2-8ºC
Transferrin, Human, Iron-Saturated (HOLO) ***	41-952-100	100 mg	2-8ºC
	41-952-500	500 mg	2-8ºC
Insulin, Human Recombinant	41-975-100	100 mg	
Basic Fibroblast Growth Factor (FGF)	30-T-218A	10µg	
	30-T-218B	50µg	

* See Chapter 5 - Serum-Free and Animal Component-Free Media
** See Chapter 2 - Classical Cell Culture Media
*** See Chapter 18 - Human Serum and Blood Products

SERUM-FREE & ANIMAL COMPONENT-FREE MEDIA

05

NutriVero™ - Serum-Free Media for Growth Proliferation and Production with Vero Cells

Serum-Free Media for mammalian Cell Culture in Suspension (e.g. Hybridoma Cells)

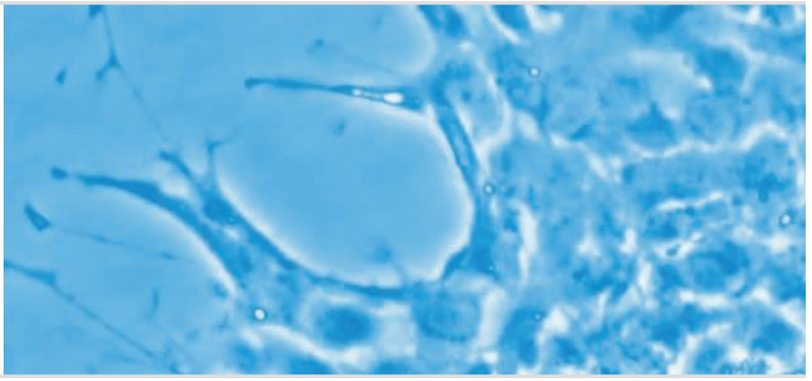
Serum-Free Media for Adherent & Suspension Cultures (e.g. CHO, Vero Cells)

Serum-Free Cell Freezing Medium

Auxiliary Solutions

Nutrimatrix™ - Extra Cellular Matrix (ECM) Coated Culture Dishes with Serum-Free Media

SERUM-FREE & ANIMAL COMPONENT-FREE MEDIA



NutriVero™ – Serum-Free Media for Growth Proliferation and Production with Vero Cells

In the light of the growing worldwide shortage and consequent price instability associated with foetal bovine serum, the development of effective serum-free medium formulations has become essential for the future growth of the biotechnology industries. Batch variation in FBS requires prior sampling of each lot. Also the use of FBS in production of biologicals causes downstream purification difficulties.

NutriVero VP1™ and NutriVero VP2™ Animal Component-Free Serum-Free Media

Product Name	Catalogue No.	Unit Size	Storage Temp.
NutriVero VP1™, Animal Component-Free Serum-Free Medium for the Monolayer Culture of Vero Cells (NutriVero VP1, ACF SFM)	05-066-1A	500ml	2-8ºC
	05-066-1B	100ml	2-8ºC
NutriVero VP2™, Animal Component-Free Serum-Free Medium for the Microcarrier Suspension Culture of Vero Cells (NutriVero VP2, ACF SFM)	05-067-1A	500ml	2-8ºC
	05-067-1B	100ml	2-8ºC

A chemically defined, animal and human component-free serum-free medium, designed to support the growth of Vero cells used in virology, virus production, and biotechnology.

There are many problems associated with the use of animal sera e.g. the fear of contamination with viral agents such as BSE, Hepatitis, HIV, BVD or other potential adventitious agents. The culture of cells in serum-free and animal component-free medium eliminates those risks. Furthermore, it allows cells to be grown under a defined set of conditions.

NutriVero VP1™ and NutriVero VP2™ are serum free, very low protein media containing no proteins or peptides of human or animal origin.
NutriVero VP1™- designed specifically for monolayer culture of Vero cells.
NutriVero VP2™- designed specifically for microcarriers suspension culture of Vero cells.
NutriVero VP1™ and NutriVero VP2™ are both suitable for large scale culturing and for growing viruses, as well as other cell culture applications, including production of recombinant proteins. The medium contains EGF and does not contain L- glutamine.

Features
- Very low protein concentration.
- No proteins or peptides of animal or human origin.
- The proteins that are used are human recombinant EGF and human recombinant Insulin.
- The formulation is without any animal origin components.
- Reduced risk of viral contamination.
- Lot to lot consistency.
- Ease of downstream product purification.

Quality Control
NutriVero VP1™ and NutriVero VP2™ are performance tested using Vero cells pre-adapted to serum-free culture in NutriVero VP1™ and NutriVero VP2™ correspondently. Additional standard evaluations are pH, osmolality and sterility tests.

Figure 1: Growth of Vero cells with NutriVero VP2™ in microcarriers suspension culture (Cytodex-1) in spinner flask; cell counting performed using crystal violet nuclei staining method.

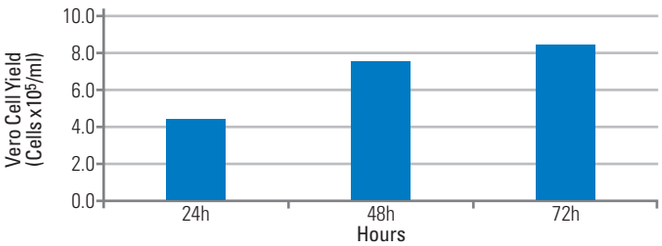
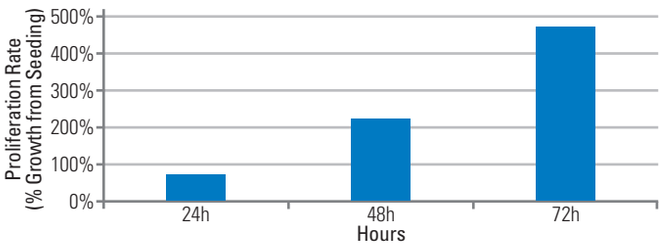
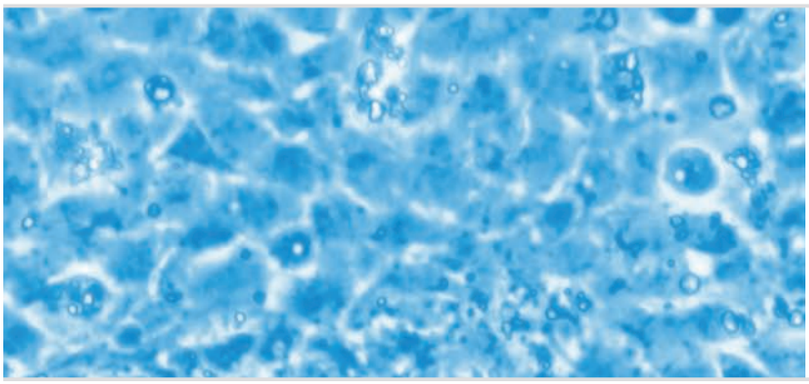
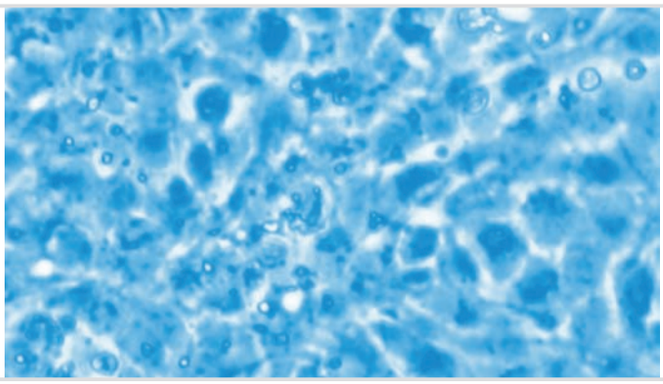


Figure 2: Growth of Vero cells with NutriVero VP2™ in Bioreactor







Serum-Free Media for Mammalian Cell Culture in Suspension (e.g. Hybridoma Cells)

Biological Industries offers a series of serum-free media products for the growth of cells in suspension.

DCCM-1, DCCM-2, LPM, BIOGRO-1 & BIOGRO-2

Product Name	Catalogue No.	Unit Size	Storage Temp.
DCCM-1 Without L-Glutamine	05-010-1A	500ml	2-8ºC
	05-010-1B	100ml	2-8ºC
DCCM-1 10X Conc. Without L-Glutamine Without Sodium Bicarbonate	05-010-5A	500ml	2-8ºC
	05-010-5B	100ml	2-8ºC
DCCM-2 Without L-Glutamine	05-015-1A	500ml	2-8ºC
	05-015-1B	100ml	2-8ºC
DCCM-2 10X Conc. Without L-Glutamine, Without Sodium Bicarbonate	05-015-5A	500ml	2-8ºC
	05-015-5B	100ml	2-8ºC
Low Protein Media BSA-Free (LPM) Without L-Glutamine	05-040-1A	500ml	2-8ºC
	05-040-1B	100ml	2-8ºC
Low Protein Media BSA-Free (LPM) 10X Conc. Without L-Glutamine, Without Sodium Bicarbonate	05-040-5A	500ml	2-8ºC
	05-040-5B	100ml	2-8ºC
BIOGRO-1 Serum-Free Medium Supplement 50X Conc.	05-600-1B	100ml	-20ºC
	05-600-1C	20ml	-20ºC
	05-600-1D	10ml	-20ºC
	05-600-1T	2ml	-20ºC
BIOGRO-2 Serum-Free Medium Supplement 50X Conc.	05-610-1B	100ml	-20ºC
	05-610-1C	20ml	-20ºC
	05-610-1D	10ml	-20ºC
	05-610-1T	2ml	-20ºC

Applications

These formulations have been successfully used in all of the following cell culture applications:

- Culture of myeloma and hybridoma cells.
- Monoclonal antibody production.
- Culture of human lymphocytes cells (including stimulated or transformed cells).

The formulations of DCCM-1 and DCCM-2 contain no growth factors and are therefore cost efficient. The relatively higher protein content of DCCM-1 is aimed at maximizing cell growth, while the lower protein content in DCCM-2 represents a compromise between cell growth promotion and easier purification in monoclonal antibody production.

LPM Medium is a formulation totally free of bovine serum albumin.
The protein content is therefore less than 18 micrograms per ml.
Despite this very low protein content LPM has proven to be very effective for the growth of a wide variety of hybridomas and other lymphocytes.

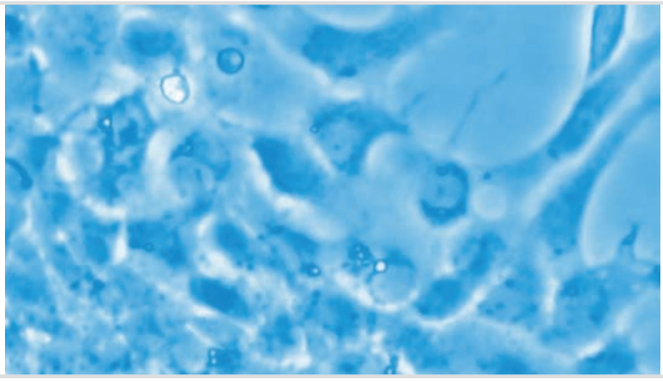
BIOGRO-1 and BIOGRO-2 are serum-free supplements intended for those customers who prefer to prepare their own final medium using a basal medium of their choice.

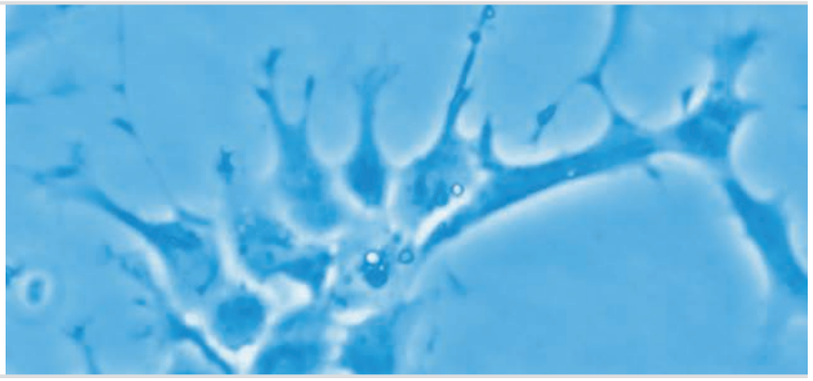
Method of Use

DCCM-1, DCCM-2 and LPM are ready-to-use media. They only require the addition of L-Glutamine and antibiotics.
The BIOGRO products are 50 fold concentrates and therefore are recommended for use at a concentration of 2% (although in some cases 1% may be sufficient). DMEM: F-12 (1:1) has been found to be most generally effective as the basal media, but many cell lines grow well with RPMI, DMEM or Iscove's.
Glutamine and antibiotics should be added to the final formulation.

Adaptation of Cells

For many cell types no adaptation procedures are necessary and may even be detrimental. In other cases standard-weaning procedures may be necessary.





Serum Free Media for Adherent & Suspension Cultures (e.g. CHO, Vero Cells)

A successful transition from cell culture work utilizing serum-containing media to serum-free cell culture often requires the use of techniques which were specifically developed for this purpose. For example, special techniques for trypsinization, neutralization of trypsin, cryopreservation of cells, as well as the use of an effective serum-free growth medium are all essential.

BIO-MPM-1, BIOCHO-1, BIOCHO-2 and BIOGRO-CHO have been successfully used in adherent and suspension cultures.

BIO-MPM-1 Multi-Purpose Serum-Free Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIO-MPM-1,Multi-Purpose SFM Without L-Glutamine	05-060-1A	500ml	2-8ºC
	05-060-1B	100ml	2-8ºC

Bio-MPM-1 is a ready-to-use serum-free medium for adherent cells, after the addition of 2 mM glutamine. The formulation contains no albumin, which has been found to be non-essential for cell growth, and even prevents efficient adhesion in some cases. The protein content of BIO-MPM-1 is therefore less than 30mg per liter, and the medium contains no growth factors or hormones other than insulin. The formulation also contains no attachment factor, which in many (but not all) cases must be added for successful use.

Adaptation of Cells

In most cases it is possible to seed the cells that have been removed from freezing medium directly in BIO-MPM-1, when the cell concentration is at least $5x10^5$ cells per $25cm^2$. The cells will begin to grow in BIOMPM-1, and after a few passages the adaptation will be complete.

However, in those cases where the cells do not adapt successfully after direct transfer, it will be necessary to perform gradual adaptation (weaning). The cells should be seeded with BIO-MPM-1 containing 5% serum and the serum concentration is then gradually reduced with each passage. The stage at which serum is completely removed is determined in the course of the weaning for each specific case.

In order to save time, we recommend parallel experiments with direct adaptation and with weaning. Generally, after the first or second passage, it will be obvious whether direct adaptation has been successful, and if not, only the weaning experiments are continued. As part of these experiments it is also necessary to test for the possible requirement for the addition of fibronectin.

After successful adaptation, it is recommended to cryopreserve the cells in Serum-Free Freezing Medium, in order to avoid the necessity of any further adaptation in the future.

Growth of Various Anchorage Dependent Cells in BIO-MPM-1 as Compared with Conventional Serum-Supplemented Medium[1]

	10% FBS			BIO-MPM-1		
Cell	Seeding density/ cm^2	Doubling time (hours)	Maximum density/ cm^2	Additives	Doubling time (hours)	Maximum density/ cm^2
3T3	$5x10^3$	24.0	$3.3x10^5$	Bombesin SBTI[3] Fibronectin	25.2	$3.3x10^5$
A-549	$1x10^4$	26.4	$4.5x10^5$	----	33.0	$2.8 x10^5$
B16-F10	$5x10^3$	30.0	$5.0x10^5$	----	30.0	$5.5 x10^5$
BGM	$1x10^4$	19.2	$4.0x10^5$	Fibronectin	30.5	$3.4 x10^5$
BHK-21	$2.5x10^4$	14.4	$4.5x10^5$	Fibronectin	12.0	$9.0 x10^5$
BS-C-1	$1x10^4$	24.0	$2.8x10^5$	----	28.0	$1.9 x10^5$
CEF	$1.2x10^4$	28.8	----	----	36.3	----
HELA	$5x10^3$	48.0	$6.5x10^5$	Fibronectin	36.0	$6.0 x10^5$
HEp-2	$5x10^3$	57.0	$5.5x10^5$	Fibronectin	30.0	$6.5 x10^5$
MA-10[2]	$2.5x10^4$	18.0	$2.7x10^5$	Fibronectin	16.5	$3.8 x10^5$
VERO	$5x10^3$	16.5	$4.1x10^5$	Fibronectin	18.0	$3.8 x10^5$

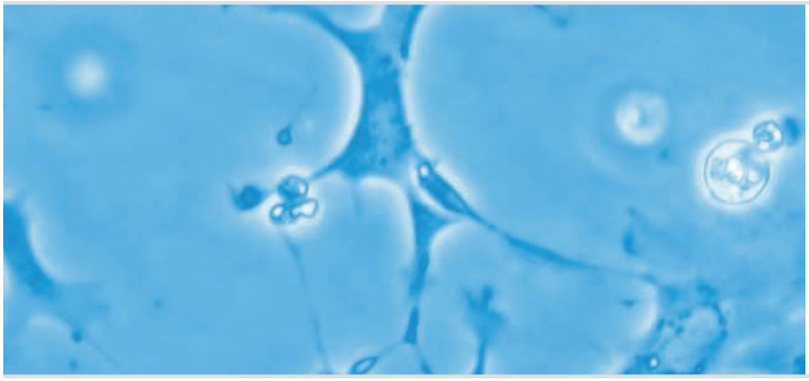
(1) MEM + 10% FBS: 3T3, A-549, BHK-21, BS-C-1, VERO.
RPMI-1640 + 10% FBS: B16-F10, BGM, HELA, HEp-2
M-199/F10 (1:2): CEF

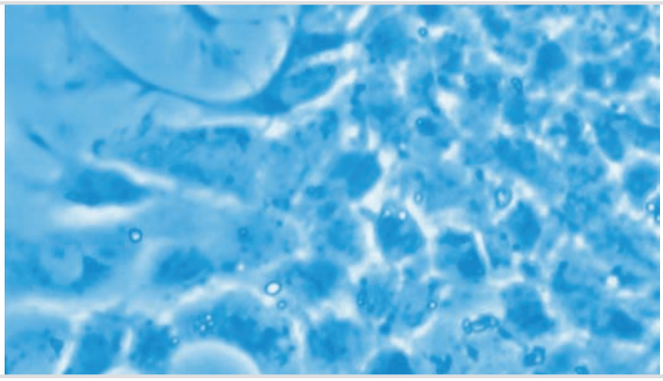
(2) Cells do not grow with FBS but with 15% horse serum in RPMI

(3) Soybean trypsin inhibitor

BIOCHO-1 Serum-Free Medium for Adherent Cultures

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOCHO-1 Serum-Free Medium Base Without L-Glutamine	05-061-1A	500ml	2-8ºC
	05-061-1B	100ml	2-8ºC
BIOGRO-CHO Serum-Free Medium Supplement 100X Conc.	05-620-1E	50ml	-20ºC
	05-620-1F	1ml	-20ºC
	05-620-1H	5ml	-20ºC





BIOCHO-1 SFM Base is the basic formulation for CHO cells. The solution contains amino acids, vitamins, salts, lipids and trace elements.
This medium is intended for the growth of CHO cells of various kinds: CHO-K1, and transfected cells containing recombinant DNA related to the DHFR gene.

BIOGRO-CHO SFM Supplement contains proteins and other components that require storage at -20°C. This product is a 100-fold concentrate. Preparation of the complete medium is carried out by adding 1% BIOGRO-CHO SFM Supplement to BIOCHO-1 SFM Base, and glutamine is then added.
The complete medium does not contain albumin, growth factors or hormones, other than insulin. Total protein concentration is less than 30mg per liter.
After preparation, the complete medium can be stored for up to 30 days at 2-8°C. Prolonged exposure to light should be avoided.

Adaptation of CHO Cells

In most cases it is possible to seed CHO cells that have been removed from freezing medium directly in the serum-free medium, when the cell concentration is at least $5x10^5$ cells per $25cm^2$. The cells will begin to grow, and after a few passages the adaptation will be complete.
However, in those cases where the cells do not adapt successfully after direct transfer, it will be necessary to perform gradual adaptation (weaning). The cells should be seeded with serum-free medium containing 5% serum and the serum concentration is then gradually reduced with each passage. The stage at which serum is completely removed is determined in the course of the weaning for each specific case.
In order to save time, we recommend parallel experiments with direct adaptation and with weaning. Generally, after the first or second transfer, it will be obvious whether direct adaptation has been successful, and if not, only the weaning experiments are continued.
After successful adaptation, it is recommended to cryopreserve the cells in Serum-Free Freezing Medium, in order to avoid the necessity of any further adaptation in the future.

BIOCHO-2 Serum-Free Medium for Suspension Cultures

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOCHO-2 Serum-Free Medium Base Without L-Glutamine	05-062-1A	500ml	2-8°C
	05-062-1B	100ml	2-8°C
BIOGRO-CHO Serum-Free Medium Supplement 100X Conc.	05-620-1E	50ml	-20°C
	05-620-1F	1ml	-20°C
	05-620-1H	5ml	-20°C

BIOCHO-2 SFM Base is the basic formulation for CHO cells. The solution contains amino acids, vitamins, salts, lipids and trace elements.
This medium is intended for the growth of CHO cells of various kinds: CHO-K1, and transfected cells containing recombinant DNA related to the DHFR gene.

BIOGRO-CHO SFM Supplement contains proteins and other components that require storage at -20°C. This product is a 100-fold concentrate.
Preparation of the complete medium is carried out by adding 1% BIOGRO-CHO SFM Supplement to BIOCHO-2 SFM Base, and glutamine is then added. The complete medium does not contain albumin, growth factors or hormones, other than insulin. Total protein concentration is less than 30mg per liter.
After preparation, the complete medium can be stored for up to 30 days at 2-8°C. Prolonged exposure to light should be avoided.

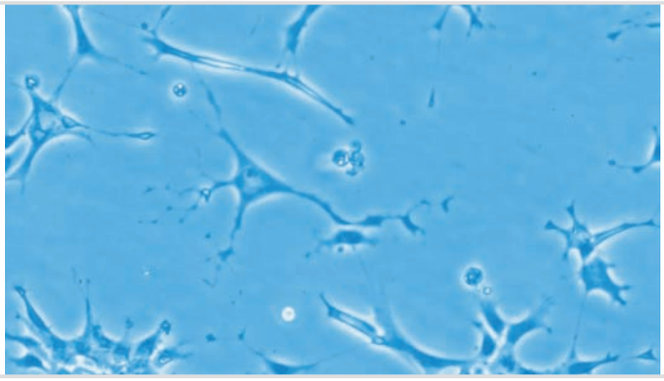
Adaptation of CHO Cells

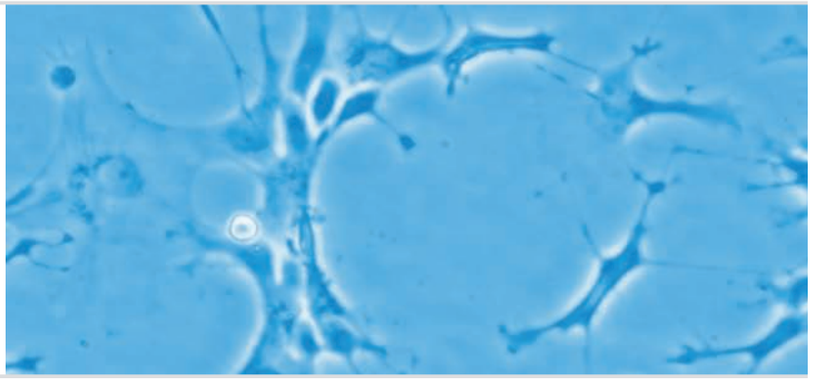
The transfer of CHO cells growing in serum-supplemented culture to serum-free suspension culture can be carried out in two ways:

1. Single Stage
   Direct transfer of the cells from serum-supplemented monolayer culture to serum-free suspension culture using BIOCHO-2 SFM Base and BIOGRO-CHO.
2. Two Stages
   Transfer of the cells from serum-supplemented monolayer culture to suspension culture using BIOCHO-2 SFM Base and 5% serum, followed in the second stage by replacement of the serum-containing media with BIOCHO-2 SFM Base and BIOGRO-CHO.

In either case, it is important to use materials and techniques that were specifically developed for serum-free culture. Following the detailed procedures given here will ensure success in the transfer to serum-free suspension culture.
As explained above, it is possible to try a single-stage approach in the transfer to serum-free suspension culture. However, sensitive recombinant cells may not adapt successfully using this approach. In any case at least 7-10 days will be required in order to reach reasonably high cell density.

In the two-stage approach, the cells are first transferred to serum-supplemented BIOCHO-2 for a period of 7 days, and the subsequent transfer to serum-free culture will require 3-4 days, or up to 10-15 days in the case of sensitive cells.





When transferring the cells to suspension culture, it is important to guarantee all of the following conditions:

Cell Concentration for seeding:	At least 500,000 per ml
Volume of the Culture:	No more than 40-50% of the volume of the spinner
Speed:	60-80 revolutions per minute
Viability of the Cells:	At least 90%

After 3-4 days the cells will begin to form aggregates of 3-4 cells, and these aggregates will then grow to contain approximately 50 cells.
After a cell density of $2x10^6$ per ml is reached, half of the culture medium should be replaced every 2 days.

Since the cells grow as aggregates, in order to follow their growth by counting, a sample must be taken while stirring is in progress; 1:1 dilution with crystalline trypsin is carried out, and the cells are left for 15 minutes at ambient temperature. After non-aggregated cells are obtained, they can be stained with Trypan Blue and counted.

Recommended Amounts of Sodium Bicarbonate and L-Glutamine to be Added in the Preparation of Single Strength Liquid Media (1x) from Concentrated Media (10x)

Product Name	Desired Product (1x) Catalogue No.	Prepared from Product (10x) Catalogue No.	Quantity Sodium Bicarbonate Solution 7.5% Catalogue No. 03-040-1 ml/Liter	Quantity L-Glutamine Solution 200mM Catalogue No. 03-020-1 ml/Liter
DCCM-1	05-010-1	05-010-5	29.4	10-20
DCCM-2	05-015-1	05-015-5	29.4	10-20
Low Protein Media BSA-Free (LPM)	05-040-1	05-040-5	29.4	10-20
BIO-MPM-1, Multi-Purpose SFM	05-060-1	05-060-5	26.9	10-20

BIOTARGET-1 Serum-Free Medium for Use with Mononuclear Cells (Lymphocytes and Monocytes)

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOTARGET-1 Without L-Glutamine	05-080-1A	500ml	2-8ºC
	05-080-1B	100ml	2-8ºC

BIOTARGET-1 has been developed specifically for use with human mononuclear cells (lymphocytes and monocytes) from peripheral blood.
In work with these cells and their sub-populations, it is critical to optimize and define the media formulation as well as pH and temperature.

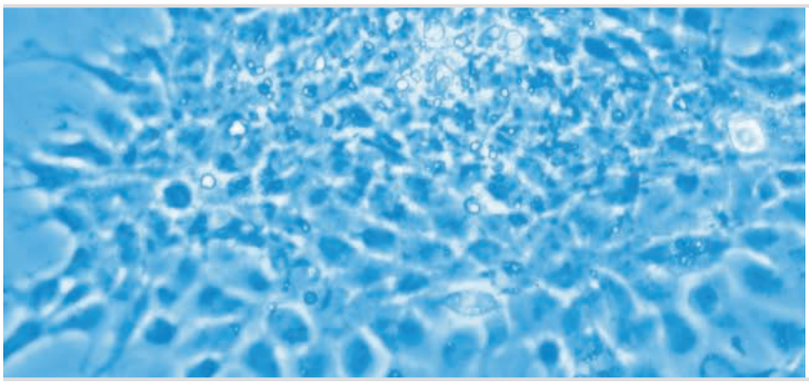
In most cases at present, these cells are grown in convential media, supplemented with human serum (A, AB) or foetal bovine serum. However, the use of serum suffers from the following disadvantages:

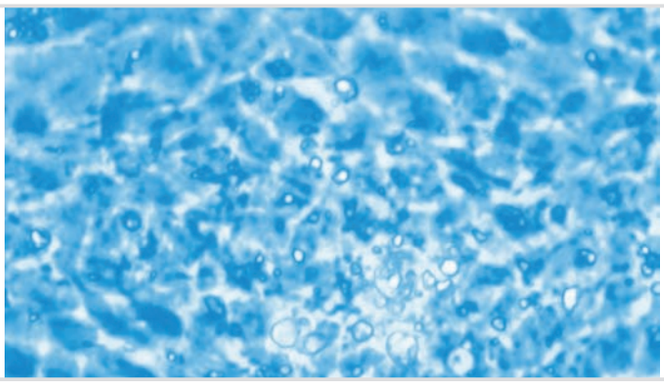
- The serum may contain non-specific growth factors, which interfere with complete activation in the desired direction.
- The serum may contain inhibitors which will limit activation of the lymphocytes.
- Lot to lot variation is certain.
- Pathogens may be introduced via the serum.
- The evaluation of the antigenic reaction, such as the quantity of the lymphokines generated, and the reaction of the lymphokines to hormones and growth factors are all more accurate in the absence of serum.

Applications for BIOTARGET-1

The applications for the use of BIOTARGET-1 are numerous and include:

1. Activation of mononuclear cells with the aid of various mitogens (PHA, CON.A, OKT-3).
2. Activation of mononuclear cells with lymphoid cells (RAJI, PEER, BA, MOLT-4, JURKAT).
3. Production of IL-2 and IL-3 from mononuclear cells.
4. Long-term culture of mononuclear cells after activation .
5. Activation of mononuclear cells with interleukin-2 in order to generate LAK or TIL cells.
6. Activation of mononuclear cells in order to generate natural killer cells (NK) .
7. Activation of mononuclear cells in order to generate cytotoxic T cells.
8. Activation of macrophages.
9. Research on the influence of various cytokines on the production of sub-populations of mononuclear cells.
10. Proliferation of the HIV virus.
11. Proliferation of retroviruses in T cells for the purposes of vaccine development





Following are several examples of the evaluation protocols by which BIOTARGET-1 was selected:

1. Mitogenic Activation of Mononuclear Cells
   Activation was evaluated with different mitogens such as PHA, CON.A and OKT-3. Proliferation was checked by measurement of the uptake of radioactive thymidine. The mitogens were added in varying concentrations and thymidine uptake was determined over several days, in order to fully evaluate the specific medium formulation.
2. Activation of Mononuclear Cells with Lymphoid Cells
   The activation of the mononuclear cells was carried out using lymphoid cells of various kinds, such as: JURKAT, RAJI, MOLT-4, and BA. Varying ratios between the tumor cells and the mononuclear cells were examined, and the proliferation was checked by measurement of the uptake of radioactive thymidine.
3. Production of Lymphokines by Activated Mononuclear Cells
   The levels of the lymphokines IL-2 and IL-3 were measured in the culture of the mononuclear cells after activation with various mitogens. IL-2 production was measured with the help of the CTLL-2 cell line. These are cytotoxic T-cells from mice, which grow only in the presence of IL-2 in the culture medium.
4. Cytotoxicity
   Mononuclear cells were seeded at a concentration of 10^6 cells per well together with RAJI cells which had been treated with mitomycin C. Varying ratios of the two cell types were examined. At the conclusion of the activation (5-7 days), the lymphocytes were collected, centrifuged, suspended in medium and seeded in microwells in order to measure proliferation and cytotoxicity. RAJI cells were labeled with radioactive chromium (10 pCi in a volume of 0.2 ml), washed three times, suspended at a concentration of 10^5 cells per ml, and divided into microwells containing the above activated lymphocytes. After 18 hours incubation, the cytolytic activity was evaluated by measuring the radioactive chromium released from the target (RAJI) cells.

BIOINSECT-1 Serum-Free Medium for Insect Cells

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOINSECT-1			
With L-Glutamine	05-050-1A	500ml	2-8°C
	05-050-1B	100ml	2-8°C

BIOINSECT-1 is a serum-free medium optimized for the culture of lepidopteran insect cells. The medium supports both suspension and stationary cultures of Sf-9 cells derived from the pupal ovarian tissue of Spodoptera frugiperda. Sf-9 cells are suitable hosts for the replication of the baculovirus Autographa colifornica nuclear polyhedrosis virus. This virus, isolated from the Alfalfa looper, is used for the recombinant expression of heterologous proteins in the baculovirus expression vector system (BEVS). Insect cells, infected with this virus, display accumulations of the highly expressed protein polyhedrin, within the nuclea (polyhedra). This protein-free medium supports the growth of Sf-9 cells with significantly better results than those obtained using TNM-FH medium (supplemented Grace's) with 10% foetal bovine serum, and production of recombinant beta-galactosidase is also excellent. BIOINSECT has showed excellent performance when cultivating high-V cells.

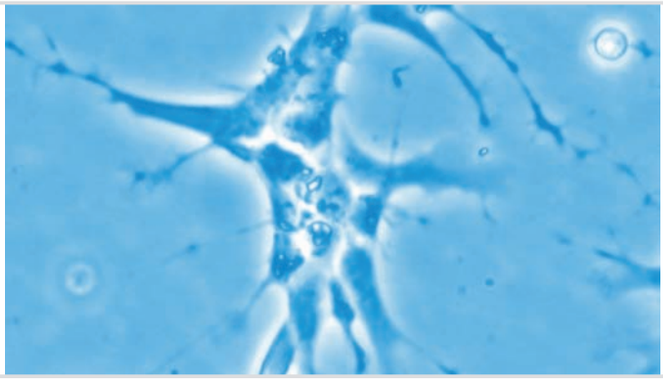
Weaning Procedure

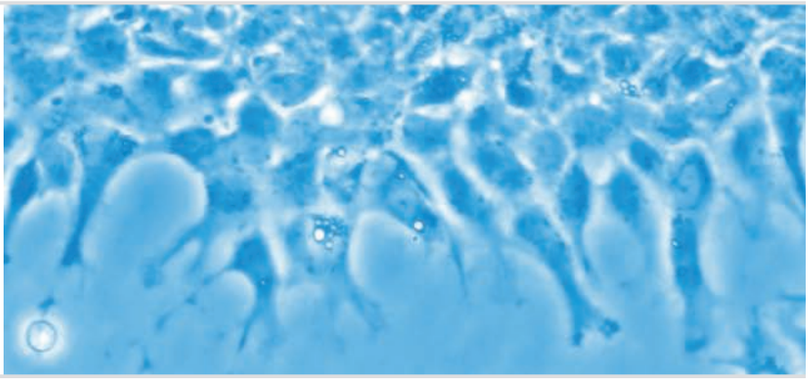
Transfer cells in the logarithmic phase from the serum-containing medium into 50% (v/v) mixture of serum-supplemented medium and BIOINSECT-1.
Subculture the cells after 3 days and reduce the percentage of the serum-supplemented medim to 40%.
Continue with the subculturing of the cells every 3 days and with each passage reduce the concentration of the serum-supplemented medium by a further 10%. On the sixth passage, the cells will be fully adapted to BIOINSECT-1 serum-free medium.

Maintenance of Sf-9 cells in BIOINSECT-1 serum-free medium:

	Stationary culture	Suspension culture
Inoculation density	6-10 x 10^4 cells/cm^2 2-3 times/week	1.5 x 10^6 cells/ml Every 3-4 days
Subculture	Subculture the cells when the viable cell count reaches 4-5 x 10^5/cm^2, with greater than 90% viability.	Subculture the cells when the viable cell count reaches 3-5 x 10^6/ml, with greater than 95% viability. After 5 days in culture, the cell density reaches 6-8 x 10^6 cells/ml.

The culture may be gently centrifuged when subculturing, in order to remove the toxic by-products in the supernatant.





Serum Free Cell Freezing Medium

Product Name	Catalogue No.	Unit Size	Storage Temp.
Serum-Free Cell Freezing Medium PF, ACF	05-065-1A	500ml	2-8ºC
	05-065-1C	20ml	2-8ºC

Protein-Free, Animal Component- Free (ACF)

When using serum-free media in mammalian cell culture, it is important to cryopreserve cells also in a medium free of serum. The novel cell freezing medium that has been developed by Biological Industries contains no serum, no proteins and no animal components but rather methylcellulose and DMSO. After freezing and thawing, a very high percentage of viable cells is obtained, and they also show excellent attachment ability as well as growth performance. In fact comparative studies have shown that in most cases higher viabilities and adhesion percentages are obtained in comparison to serum-containing freezing medium. Therefore, the use of this serum-free freezing medium is also recommended for cell culture employing serum-supplemented growth media.

Performance Validation

Serum-free Freezing Medium is a complete, ready to use solution which is designed to protect frozen cells in liquid nitrogen for long-term storage, without any use of protein or other animal components.

1. Materials and methods

1.1 Cell lines

Various cells grown under serum-free conditions were frozen with serum-free freezing medium and with freezing medium containing serum.

1.2 Freezing method

Serum-free Freezing Medium (Cat. no.: 05-065-1) containing 10% DMSO and Methylcellulose and basal medium containing 10% DMSO and 20% FBS were used as freezing media. The cells were frozen in the appropriate freezing medium in a concentration of 3-5x10^6 per ml. One ml of these cell suspensions was transferred to a plastic ampoule and frozen by decreasing the temperature at a rate of 1-2°C/min. The ampoules were kept in liquid nitrogen until tested.

1.3 Cell recovery measurements

When thawing, the frozen ampoules were put in a water bath at 37°C. After dilution with culture medium and centrifugation, the cells were resuspended with either serum-free medium or medium containing serum. Viability of cells was determined by the trypan blue dye exclusion method. Adhesion of cells was determined by counting the attached cells only 6-24 hours after culture of the cells.

2. Results

2.1 Freezing of cells in Serum-free Freezing Medium in comparison to freezing medium containing serum:

Table 1: Thawing of cells 24 hours after freezing in liquid nitrogen

	Viability %		Adhesion %	
Cell	Serum-free Freezing Medium	Freezing Medium Containing Serum	Serum-free Freezing Medium	Freezing Medium Containing Serum
3T3	85	83	100	83
BGM	91	83	88	88
VERO	66	71	62	33
HEp-2	75	69	100	92
BSC-1	82	77	22	10

2.2 Long term storage of cells in liquid nitrogen using Serum-free Freezing Medium.

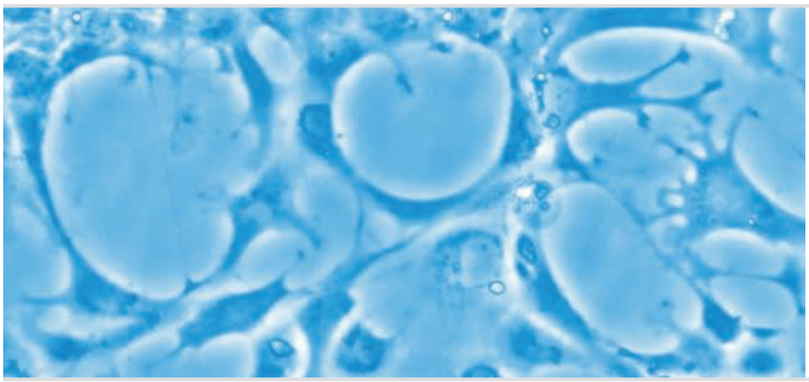
Table 2: Recovery of cells frozen in Serum-free Freezing Medium

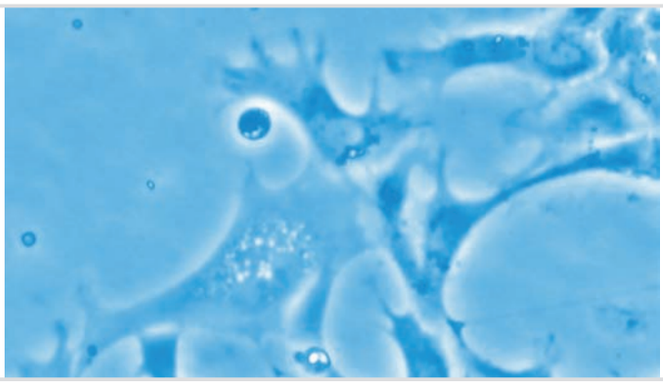
	24 hours storage		6 months storage		4 years storage	
Cell	Viability	Adhesion	Viability	Adhesion	Viability	Adhesion
B16-F10	85	100	74	79	72	80
BGM	80	70	61	100	66	92
BHK-21	80	93	64	100	71	95
CHO-DHFR	70	70	69	81	70	73
HELA	87	78	70	90	80	83
HEp-2	90	100	63	100	66	94
MA-10	88	95	81	100	83	91
VERO	90	94	71	68	73	76

3. Summary

The Serum-free Freezing Medium supports efficient cryopreservation of various cell lines cultured in serum-free media.

After freezing and thawing, a very high percentage of viable cells is obtained, and they also show excellent attachment ability as well as growth performance. In fact, the present study has shown that in most cases higher viabilities and adhesion percentages are obtained in comparison to freezing medium containing serum. Therefore, the use of this Serum-free Freezing Medium is also recommended for cell culture employing serum-supplemented growth media.





Method of use

It is recommended to detach the adherent cells (to be frozen) with crystalline trypsin solution, and neutralization with Soybean Trypsin Inhibitor Solution. After centrifuging, suspend the cells in cold serum-free freezing medium at a concentration of 3-5 million cells per ml. Freeze the cells gradually (1-2°C per minute) and store them in liquid nitrogen.

Thawing should be performed at 37°C. Immediately after thawing, suspend the cells in serum-free growth medium at a ratio of at least 1:10. Then centrifuge and suspend at high concentration in growth medium.

Auxiliary Solutions

Cell Dissociation Solution (Non-Enzymatic)

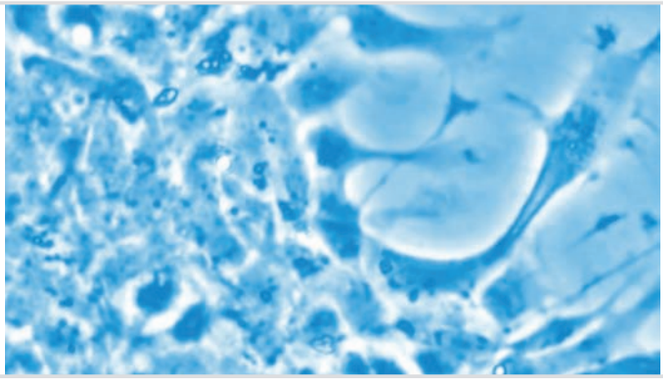
Product Name	Catalogue No.	Unit Size	Storage Temp.
Cell Dissociation Solution (non-enzymatic)	03-071-1B	100ml	2-8ºC

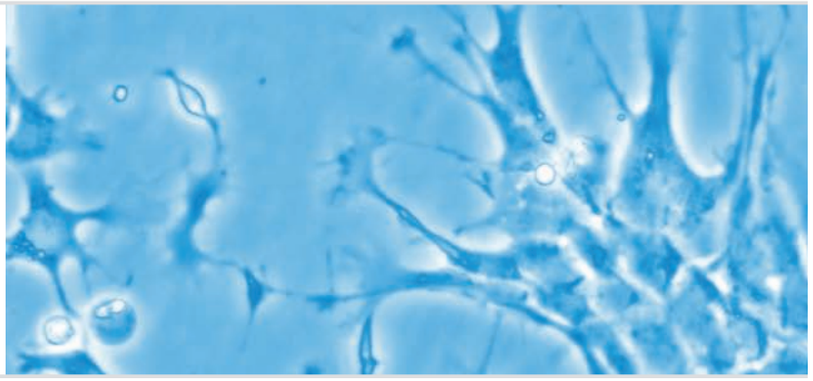
Cell Dissociation Solution is a special, non-enzymatic formulation with a proprietary mixture of chelators for gently dislodging adherent cell types from culture vessels. Cell Dissociation Solution helps to maximize the yield of functionally viable cells from these culture vessels. It is a non-enzymatic, protein-free and animal component-free solution. Another major advantage is that cells can be exposed to this solution for longer periods of time without the risk of subjecting them to protein digestive enzymes such as trypsin. However, the solution is not recommended for cells with very adhesive properties. For those cell lines which are difficult to dislodge, Biological Industries has developed a Papain Dissociation Solution.

Features

Contains a proprietary mixture of chelators. Contains no enzymes or proteases.

- Works with serum-free and serum-containing media.
- Reduces the risk of cell damage associated with trypsin.
- Chemically defined.
- Contains no products of animal origin.
- Supplied as a ready-to-use solution.





Papain Dissociation Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Papain Dissociation Solution	03-072-1B	100ml	-20ºC

Papain is a nonspecific, endolytic, sulfhydrl protease or protein-cleaving enzyme, known as cysteine-endopeptidase, and is derived and isolated from papaya fruit (i.e. Carica papaya). More specifically, it is isolated from the papaya latex, which is then utilized in a wide variety of applications. Papain is commonly used in cell isolation procedures, where it has proven to be more efficient and less destructive than other proteases on certain tissues such as and including, among others, the dissociation of retinal neurons[1], in the preparation of primary neurons from the visual cortex of postnatal rats[2], and for the isolation of smooth muscle cells[3].
Papain has a wide specificity in that it will degrade most protein substrates more extensively than the pancreatic proteases and has been proven not only to manifest fewer untoward and negative ramifications producing less cell and tissue trauma, but also to be much more effective than other available proteases. Biological Industries' Papain Dissociation Solution is a ready-to-use solution and is one of our non-animal alternatives for trypsin.

Physical Properties and Kinetics
Papain is a cysteine protease hydolase enzyme of the peptidase C1 family derived from the papaya family, Carica papaya and the mountain papaya, Vasconcellea cundinamarcensis. It consists of a single peptide chain with three disulfide bridges and a sulfhydrl group necessary for the activity of the enzyme.

Specificity
Papain is more effective in digesting most protein substrates more extensively and effectively than pancreatic proteases. It further exhibits broad specificity cleaving peptide bonds of such basic amino acids as leucine and glycine. In addition to the aforementioned activity, it also hydrolyzes esters and amides.

(1) Shen J., et al., Japanese Journal of Physiology, 1995
(2) Huettner, J.E. Baughman, R.W., Journal Of Neuroscience, 1986
(3) Kinoshita, K. et.al., American Journal of Physiology, Gastrointestinal and Liver Physiology, 2003 and Driska, S.P. et.al., Journal of Applied Physiology, 1999.

Bovine Fibronectin Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Fibronectin Solution (Bovine), 1mg/ml	03-090-1-01	1ml	2-8ºC
	03-090-1-05	5ml	2-8ºC

Fibronectin is an attachment factor that facilitates the attachment and cytoplasmic spreading of all types of anchorage-dependent cells. Fibronectin is particularly useful for the culture of cells that are not capable of synthesizing their own biomatrix, or when culturing cells in serum-free medium.

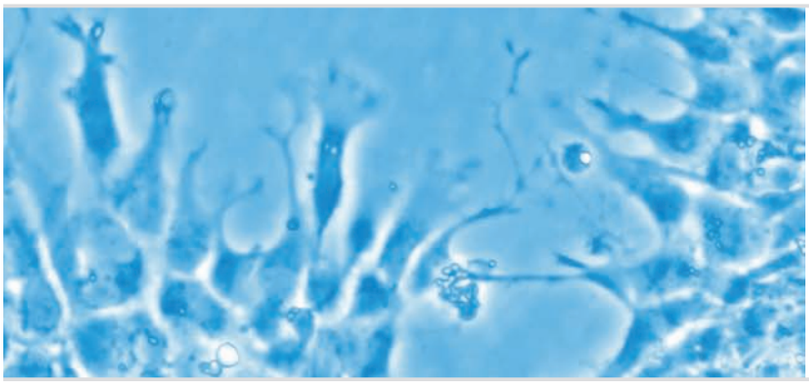
Suggested Coating Procedures
The Fibronectin should be added to the growth medium in the growth vessel, which is then placed in an incubator 30-60 minutes before seeding. The recommended concentration of the Fibronectin is 5 micrograms per ml of medium. When the medium is replaced in the days following initial seeding, no further Fibronectin is required.

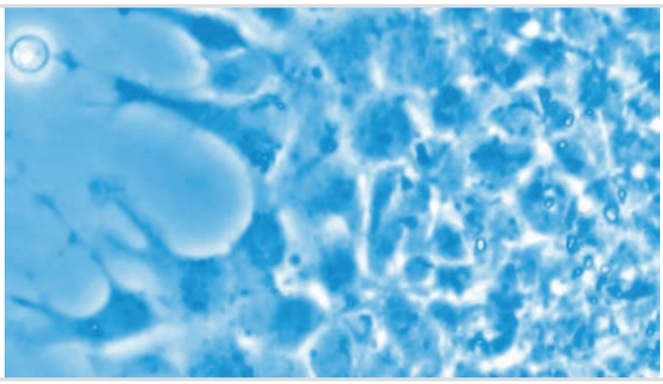
Crystalline Trypsin Solution & Soybean Trypsin Inhibitor Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Crystalline Trypsin Solution (0.02%) Without Phenol Red	03-047-1A	500ml	-20ºC
	03-047-1B	100ml	-20ºC
Soybean Trypsin Inhibitor 50X Conc., 5mg/ml	03-048-1C	20ml	-20ºC

Crude trypsin is often the subculturing agent of choice for cell dissociation/disaggregation of adherent cells, although the treatment may be cytotoxic if prolonged. Over-trypsinization is a common cause of subculture problems. Regarding the use of crude trypsin, some important facts must be noted:
- Cells must NEVER remain in the crude trypsin for longer than 3-5 minutes as they may be seriously damaged in the process (i.e. damage to the intracellular proteins).
- Cells should NEVER be left without a fluid layer.

The use of crystalline trypsin, rather than crude trypsin, most often performs better long-term cell growth in serum-free medium formulations. It is specifically formulated to have a gentle nature with much better cell viability, in which the cells are not subject to the vagaries of time and circumstance as when the cruder forms of trypsin are utilized.





Some of the advantages of crystalline trypsin versus the cruder trypsin forms:

1. Crystalline trypsin does not damage cells after prolonged exposure.
2. Crystalline trypsin does not require multiple-change procedures and thus is less labor-intensive.
3. Crystalline trypsin maintains better cell viability and enhances the process of cell passaging.
4. Crystalline trypsin is not as cytotoxic to cells with all the negative ramifications of crude trypsin.
5. Biological Industries' Crystalline Trypsin Solution also contains additives that protect the cell wall, enhancing cell viability.

In a serum-free culture environment, the cells must be separated by rapid centrifugation or by utilizing trypsin inhibitors such as Soybean Trypsin Inhibitor (SBTI). SBTI is a single polypeptide that forms a stable, stoichiometric, enzymically inactive complex with trypsin, thereby reducing the availability of trypsin by somewhat binding chymotrypsin. With Biological Industries' Soybean Trypsin Inhibitor Solution, any excess Crystalline Trypsin Solution may be completely neutralized, thereby avoiding the use of serum for this purpose. The cells may then be re-suspended successfully in a suitable growth medium.

The use of animal-derived components in Biopharmaceutical Manufacturing is experiencing ever-increasing regulatory scrutiny. Therefore, there is the need to develop non-animal source products for cell culture. Trypsin is an essential product for cell culture manipulation. However, it is purified from animal-source materials with one unfortunate notable disadvantage: contamination from variegated sources such as viruses, other potential adventitious agents and other unwanted enzymes.

Accutase Solution, primary human cell culture tested

Product Name	Catalogue No.	Unit Size	Storage Temp.
Accutase Solution, primary human cell culture tested	03-073-1B	100ml	-20ºC

Accutase is an alternative cell detachment solution to trypsin and can also be used for tissue dissociation. It is a ready to use solution and was developed for very gentle and effective detachment of adherent cells. The well balanced combination of protease and collagenolytic activities ensures that surface proteins and epitopes stay entirely intact. This makes it perfectly suited for applications, which require unchanged surface conditions.

Nutrimatrix™ - ECM Coated Culture Dishes with Serum-Free Media

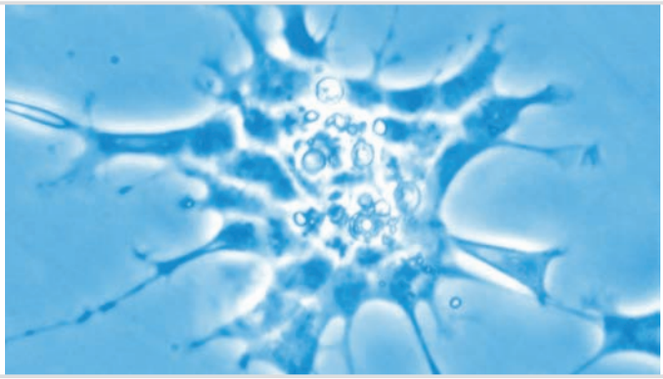
Coated with Extracellular Matrix (ECM); Simulates In Vivo Conditions

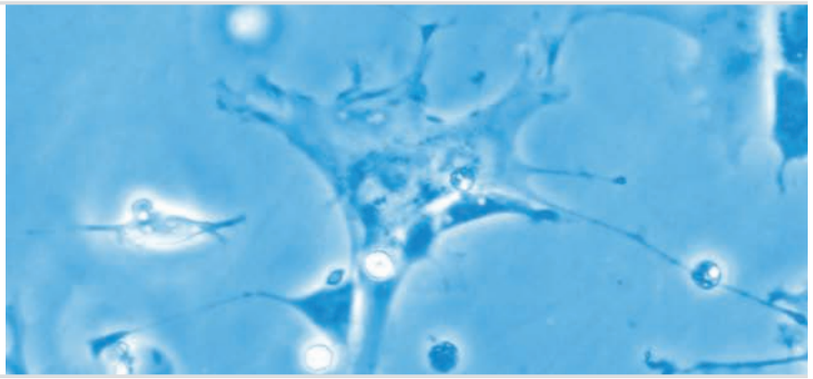
Cultured endothelial cells secrete large amounts of extracellular matrix (ECM) onto the plastic surface during their In Vitro proliferation.
This ECM is similar in its chemical composition and organization to naturally occurring basement membranes upon which cells migrate, proliferate and differentiate In Vivo. Thus, disposable plastic ware coated with ECM mimics the In Vivo environment under In Vitro experimental conditions.
Cells placed in contact with ECM attach rapidly, exhibit high plating and cloning efficiencies, proliferate rapidly, reach a high saturation density, exhibit lower requirements for growth factors and have better plating consistency.

Nutrimatrix™ ECM- coated dishes with serum-free media support the maintenance and normal function of hormone secreting cells such as pancreatic islet cells, hepatocytes pituitary cells, granulosa cells, etc. The ability to culture cells from endocrine organs makes it possible to study control mechanisms of hormone production.
The ECM/serum- free medium combination promotes research possibilities on various cellular products due to the following multiple effects:

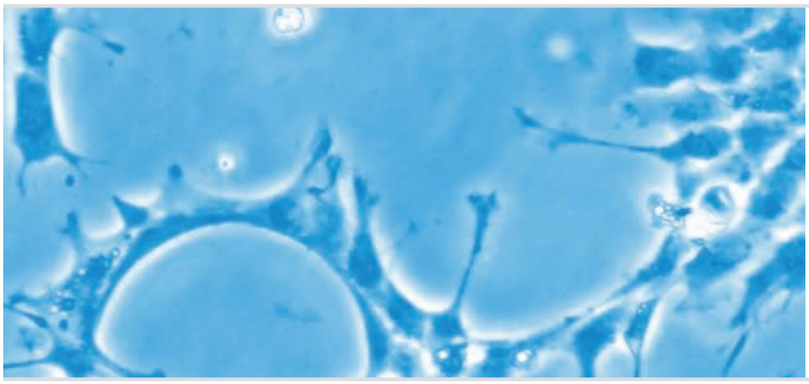
- Inducing differentiation: Various cells do not maintain their differentiated functions outside the body and therefore do not produce and/or secrete active materials which are produced in vivo. Since secretory cells are mainly epithelial, contact with a basement membrane during growth is required for expression of differentiated functions.
  The development of appropriate conditions for the maintenance of differentiated cells in culture is a key to study specific functions of hormone secreting cells and for increasing the yield of secreted products.
- Suppressing fibroblasts: Serum-free media have been shown to suppress the growth of fibroblasts and hence allow the maintenance of almost pure epithelial cell cultures.
- Purification: In many cases it is difficult to separate the secreted material from the various proteins present in serum. Since ECM allows the growth of cells in serum-free media, it facilitates the purification of various cellular products from medium lacking most macromolecular contaminants.
- Increasing yields: In many instances the minute quantities of materials secreted by cultured cells is a major drawback in the production of cellular materials. Use of serum-free medium presents exciting possibilities for increasing the yield of various cellular products through batch processing methods.

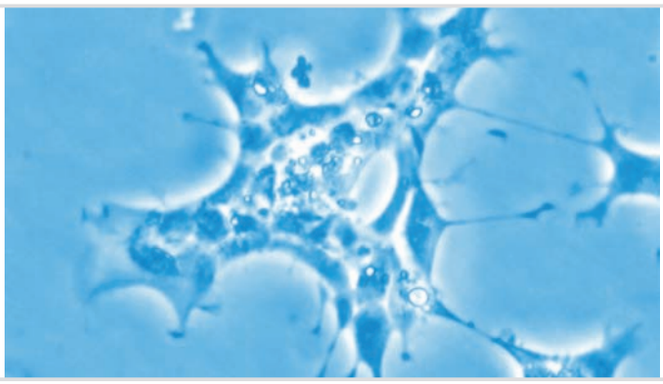
For the list of Nutrimatrix™ items and more information see chapter 7.





Product Name	Catalogue No.	Unit Size	Storage Temp.
NutriVero – Serum-Free Media for Growth Proliferation and Production with Vero Cells			
NutriVero VP1™, Animal Component-Free Serum-Free Medium for the Monolayer Culture of Vero Cells (NutriVero VP1, ACF SFM)	05-066-1A	500ml	2-8ºC
	05-066-1B	100ml	2-8ºC
NutriVero VP2™, Animal Component-Free Serum-Free Medium for the Microcarrier Suspension Culture of Vero Cells (NutriVero VP2, ACF SFM)	05-067-1A	500ml	2-8ºC
	05-067-1B	100ml	2-8ºC
Serum-Free Media for Mammalian Cell Culture in Suspension (e.g. Hybridoma Cells)			
DCCM-1 without L-Glutamine	05-010-1A	500ml	2-8ºC
	05-010-1B	100ml	2-8ºC
DCCM-1 10X Conc., Without L-Glutamine Without Sodium Bicarbonate	05-010-5A	500ml	2-8ºC
	05-010-5B	100ml	2-8ºC
DCCM-2 without L-Glutamine	05-015-1A	500ml	2-8ºC
	05-015-1B	100ml	2-8ºC
DCCM-2 10X Conc., Without L-Glutamine, Without Sodium Bicarbonate	05-015-5A	500ml	2-8ºC
	05-015-5B	100ml	2-8ºC
Low Protein Medium BSA-Free (LPM), Without L-Glutamine	05-040-1A	500ml	2-8ºC
	05-040-1B	100ml	2-8ºC
Low Protein Medium BSA-Free (LPM) 10X Conc., Without L-Glutamine, Without Sodium Bicarbonate	05-040-5A	500ml	2-8ºC
	05-040-5B	100ml	2-8ºC
BIOGRO-1 SFM Supplement 50X Conc.	05-600-1B	100ml	-20ºC
	05-600-1C	20ml	-20ºC
	05-600-1D	10ml	-20ºC
	05-600-1T	2ml	-20ºC
BIOGRO-2 SFM Supplement 50X Conc.	05-610-1B	100ml	-20ºC
	05-610-1C	20ml	-20ºC
	05-610-1D	10ml	-20ºC
	05-610-1T	2ml	-20ºC
Serum-Free Media for Adherent & Suspension Cultures (e.g. CHO, Vero Cells)			
BIOINSECT-1, With L-Glutamine	05-050-1A	500ml	2-8ºC
	05-050-1B	100ml	2-8ºC





Product Name	Catalogue No.	Unit Size	Storage Temp.
BIO-MPM-1,Multi-Purpose SFM, Without L-Glutamine	05-060-1A	500ml	2-8ºC
	05-060-1B	100ml	2-8ºC
BIOCHO-1 SFM Base Without L-Glutamine	05-061-1A	500ml	2-8ºC
	05-061-1B	100ml	2-8ºC
BIOCHO-2 SFM Base Without L-Glutamine	05-062-1A	500ml	2-8ºC
	05-062-1B	100ml	2-8ºC
BIOTARGET-1 without L-Glutamine	05-080-1A	500ml	2-8ºC
	05-080-1B	100ml	2-8ºC
BIOGRO-CHO SFM Supplement 100X Conc.	05-620-1E	50ml	-20ºC
	05-620-1F	1ml	-20ºC
	05-620-1H	5ml	-20ºC
Serum-Free Cell Freezing Medium			
Serum-Free Cell Freezing Medium	05-065-1A	500ml	2-8ºC
	05-065-1C	20ml	2-8ºC
Auxiliary Solutions			
Crystalline Trypsin Solution (0.02%), Without Phenol Red	03-047-1A	500ml	-20ºC
	03-047-1B	100ml	-20ºC
Soybean Trypsin Inhibitor 50X Conc., 5mg/ml	03-048-1C	20ml	-20ºC
Cell Dissociation Solution (non-enzymatic)	03-071-1B	100ml	2-8ºC
Papain Dissociation Solution	03-072-1B	100ml	-20ºC
Fibronectin Solution (Bovine), 1mg/ml	03-090-1-01	1ml	2-8ºC
	03-090-1-05	5ml	2-8ºC
Accutase Solution, primary human cell culture tested	03-073-1B	100ml	-20ºC
NutriStem™ Xeno-Free Serum-Free Medium for Human Embryonic Stem Cells*			
NutriStem™ hESC XF Xeno-Free medium for hESC With HSA	05-100-1A	500ml	-20ºC
	05-100-1B	100ml	-20ºC
AF NutriStem™ hESC XF Xeno-Free medium for hESC Without HSA	05-102-1A	500ml	-20ºC
	05-102-1B	100ml	-20ºC
Human Serum Albumin (HSA Solution, 10%), Optimized for Human Embryonic Stem Cells (hESC)	05-720-1B	100ml	-20ºC
	05-720-1E	50ml	-20ºC

* See Chapter 4 - Stem Cells products

REAGENTS, SUPPLEMENTS & BIOCHEMICALS

06

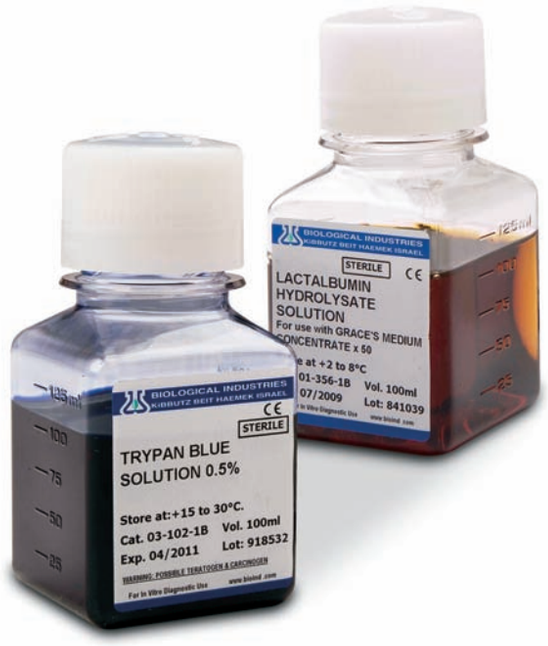
REAGENTS, SUPPLEMENTS & BIOCHEMICALS

Reagents and Supplements

Biological Industries' reagents and supplements are specifically designed for cell culture allowing for the growth and propagation of a wide spectrum of cell types under controlled conditions. In vitro cell culture systems provide the researcher with the appropriate means for effectively studying cell growth and differentiation, in order to understand the cellular response to specific environmental stimuli. Each and every cell culture system is designed to meet its nutritional and metabolic niche requirements once a basic medium is chosen. In order to realize maximum yields, the cell culture must have a large reserve of not only the basic nutrients, but also the essential requirements which provide an energy source, amino acids, vitamins and other various supplementations to enhance cell growth and performance. Biological Industries' reliable and proven products will help you reach your goals.

Biological Industries' products are not only well-known in many medical centers and hospitals nationally and internationally, but also in many prominent and renowned research laboratories in such diverse fields as cell culture and biology, immunology and oncology, virology, microbiology and parasitology, as well as in vitro fertilization among many other specialized fields. Customized solutions and media formulations are manufactured to exact specifications under strict and rigorous Quality Assurance/Quality Control Guidelines and supplied to numerous biotech and biopharmaceutical firms and corporations, from small-volume to large-scale operations, in order to meet your needs and requirements. All media are performance tested with applicable documentation that meets international standards, in order to ensure lot-to-lot uniformity and the highest quality. Bulk pricing is available upon request.

The following is a list of reagents and supplements that all undergo extensive and vigorous Quality Control Protocols.



Product Name	Concentration	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
BME Amino Acids Solution Without L-Glutamine	100X	01-315-1B	100ml	2-8ºC	135-136
BME Vitamins Solution	100X	01-316-1B	100ml	-20ºC	135-136
MEM Amino Acids Solution Without L-Glutamine	50X	01-325-1B	100ml	2-8ºC	
MEM Vitamins Solution	100X	01-326-1B	100ml	-20ºC	135-136
MEM Non-Essential Amino Acids Solution	100X	01-340-1B	100ml	2-8ºC	135-136
Ribonucleosides and Deoxyribonucleosides for MEM-Alpha	500X	01-343-1D	10ml	-20ºC	129
Lactalbumin Hydrolysate Solution, 166.6 gr/liter	50X	01-356-1B	100ml	2-8ºC	
Yeastolate Solution, 166.6 gr/liter	50X	01-357-1B	100ml	2-8ºC	
Human Recombinant Insulin Solution (3.7 mg/ml)	100 Units/ml	01-818-1H	5ml	2-8ºC	
Gelatin Solution*	0.1%	01-944-1A	500ml	2-8ºC	
		01-944-1B	100ml	2-8ºC	
Bovine Albumin Solution, Fraction V in saline	10%	03-010-1B	100ml	-20ºC	
Ethylenediaminetetraacetic Acid (EDTA) Disodium Salt Solution, in DPBS	0.05%	03-015-1B	100ml	AMB	
L-Glutamine Solution, 29.2mg/ml in Saline	200mM	03-020-1A	500ml	-20ºC	
		03-020-1B	100ml	-20ºC	
		03-020-1C	20ml	-20ºC	
L-Alanyl L-Glutamine (Stable Glutamine)**	200 mM	03-022-1B	100ml	-20ºC	
		03-022-1C	20ml	-20ºC	
HEPES Buffer Solution, pH 7.3 at 37ºC	1M	03-025-1B	100ml	AMB	
		03-025-1C	20ml	AMB	
Sodium Bicarbonate Solution	7.5%	03-040-1A	500ml	AMB	
		03-040-1B	100ml	AMB	
Sodium Bicarbonate Solution	5%	03-041-1A	500ml	AMB	
		03-041-1B	100ml	AMB	
Sodium Pyruvate Solution, 11.0mg/ml	100mM	03-042-1B	100ml	-20ºC	
Water, Cell Culture Grade		03-055-1A	500ml	AMB	
SPGA Solution		03-060-1A	500ml	-20ºC	
HAT Supplement, (Hypoxanthine 680.5mg/l, Aminopterin 8.81mg/l, Thymidine 193.8mg/l), in DPBS	50X	03-080-1B	100ml	-20ºC	
		03-080-1C	20ml	-20ºC	
HT Supplement (Hypoxanthine 680.5mg/l, Thymidine 193.8mg/l), in DPBS	50X	03-085-1B	100ml	-20ºC	
		03-085-1C	200ml	-20ºC	
Fibronectin Solution (Bovine)***	1mg/ml	03-090-1-01	1ml	2-8ºC	
		03-090-1-05	5ml	2-8ºC	
Phenol Red Solution, in DPBS	5mg/ml	03-100-1B	100ml	AMB	
Trypan Blue Solution, in Saline****	5mg/ml	03-102-1B	100ml	AMB	

* See Chapter 4 - Mouse Embryonic Stem Cells
** See Chapter 2 - Classical Cell Culture Media
*** See Chapter 7 - Attachment Factors
**** See Chapter 15 - Cell Viability

Cell Culture-Tested Biochemicals

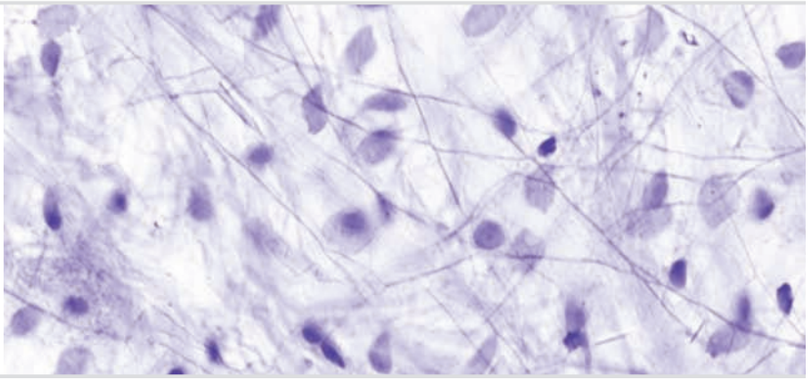
Product Name	Catalogue No.	Unit Size
L-Alanine	41-239-25	25 gr
	41-239-100	100 gr
L-Arginine Free Base	41-219-25	25 gr
	41-219-100	100 gr
L-Arginine Hydrochloride	41-201-25	25 gr
	41-201-100	100 gr
L-Asparagine Monohydrate	41-215-25	25 gr
	41-215-100	100 gr
L-Aspartic Acid	41-216-25	25 gr
	41-216-100	100 gr
L-Cysteine Hydrochloride Hydrate	41-241-25	25 gr
	41-241-100	100 gr
L-Cystine	41-223-25	25 gr
	41-223-100	100 gr
L-Glutamic Acid	41-217-25	25 gr
	41-217-100	100 gr
L-Glutamine	41-218-25	25 gr
	41-218-100	100 gr
Glycine	41-202-25	25 gr
	41-202-100	100 gr
L-Histidine Hydrochloride Monohydrate	41-203-25	25 gr
	41-203-100	100 gr
L-Isoleucine	41-204-25	25 gr
	41-204-100	100 gr
L-Leucine	41-205-25	25 gr
	41-205-100	100 gr
L-Lysine Monohydrochloride	41-206-25	25 gr
	41-206-100	100 gr
L-Methionine	41-207-25	25 gr
	41-207-100	100 gr
L-Phenylalanine	41-208-25	25 gr
	41-208-100	100 gr
L-Proline	41-221-25	25 gr
	41-221-100	100 gr
L-Serine	41-209-25	25 gr
	41-209-100	100 gr
L-Threonine	41-210-25	25 gr
	41-210-100	100 gr
L-Tryptophan	41-211-25	25 gr
	41-211-100	100 gr
L-Tyrosine	41-222-25	25 gr
	41-222-100	100 gr
L-Valine	41-212-25	25 gr
	41-212-100	100 gr
Bovine Serum Albumin Fraction V	41-903-25	25 gr
	41-903-100	100 gr
EDTA Disodium Dihydrate	41-922-25	25 gr
	41-922-100	100 gr
Gentamycin Sulfate	41-503-1	1 gr
	41-503-5	5 gr
Glucose Anhydrous	41-302-500	500 gr
Hepes	41-122-25	25 gr
	41-122-100	100 gr
Insulin, Human Recombinant	41-975-100	100 mg
Kanamycin Sulfate	41-507-1	1 gr
	41-507-5	5 gr
MOPS	41-811-50	50 gr
	41-811-100	100 gr
Neomycin Sulfate	41-505-1	1 gr
	41-505-5	5 gr
Nystatin	41-506-1	1 gr
	41-506-5	5 gr
Penicillin G Sodium	41-501-10	10gr
	41-501-25	25 gr
	41-501-100	100 gr
Streptomycin Sulfate	41-502-25	25 gr
	41-502-100	100 gr
Collagen Type I, Rat Tail	41-843-25	25mg
	41-843-100	100mg
Transferrin, Human, Substantially Iron-Free	41-951-100	100 mg
	41-951-500	500 mg
Transferrin, Human, Iron-Saturated	41-952-100	100 mg
	41-952-500	500 mg
Trypsin, Porcine Pancreas (1:250)	41-920-25	25 gr
	41-920-100	100 gr

These chemicals are regularly being used by Biological Industries for manufacturing of cell culture products and can be purchased separately.

ATTACHMENT FACTORS

07

ATTACHMENT FACTORS



Attachment factors are structural proteins or protein-like substances that have adherent capabilities and increase cell-substrate interactions in a culture dependent attachment milieu. A number of glycoproteins have been identified that promote and/or influence in vitro cell attachment to the surface or substratum of the culture vessel.

Normal attachment, growth and development of many cell types are dependent on attachment factors and extracellular matrix components. While some cells are able to synthesize these components, others require an exogenous source, particularly when grown in serum-free culture.

The growth and differentiation of anchorage-dependent cells are often strongly influenced by either glass or plastic culture flasks utilized as a substrate. In order to facilitate attachment, cell spreading, growth, morphology, differentiation, and motility of your cells, Biological Industries offers an extensive line of attachment and matrix factors. Each lot is cell culture tested to assess its ability to promote cell attachment and spreading.

Collagen is a major structural protein of extracellular matrix and is the principal protein found in connective tissues. It is found not only in the organic portion of bones, skin, teeth and tendons, but also occurs in other parts of the body as fibrous inclusions. Like other fibrous proteins, collagen is not readily available unless it undergoes heat treatment such as boiling which converts collagen into gelatin. It is an unusual protein, rich in amino acids such as glycine, lysine, proline and others but unfortunately not enough of the essential amino acids. Usually the gelatin derived from collagen is a relatively poor-quality protein.

Gelatin solution (0.1%) is intended for coating cell culture flasks or plates utilized in the growth of Mouse ES cells without a feeder layer. Leukemia Inhibitory Factor (LIF), a pleiotropic, polyfunctional glycoprotein (IL-6) cytokine, should be added to the medium. This impacts growth promotion and prevents cell differentiation on a wide array of various tissue types and target cells.

Bovine Fibronectin Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Bovine Fibronectin Solution 1mg/ml	03-090-1-01	1ml	2-8ºC
	03-090-1-05	5ml	2-8ºC

Fibronectin is an attachment factor that facilitates the attachment and cytoplasmic spreading of all types of anchorage-dependent cells. Fibronectin is particularly useful for the culture of cells that are not capable of synthesizing their own biomatrix or when culturing cells in serum-free medium.

Source
Irradiated citrated bovine plasma.

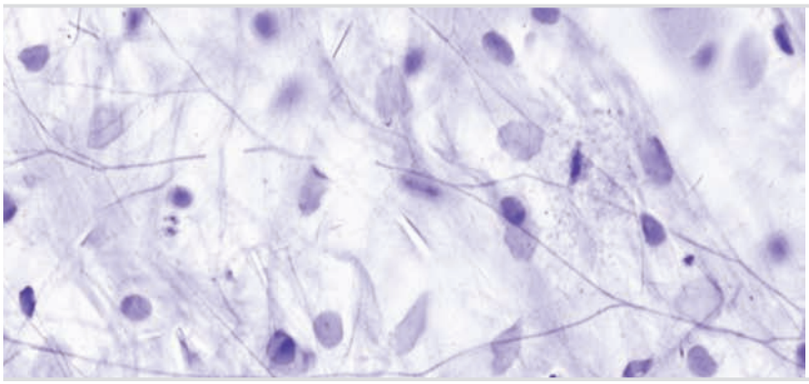
Description
A clear sterile solution containing Fibronectin, obtained by affinity purification on gelatin-sepharose from bovine plasma. The Fibronectin solution contains buffer salts.

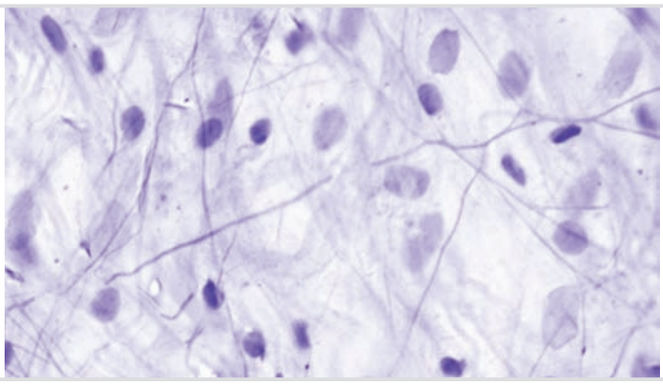
Concentration
1mg/ml, based on E (1%, 280nm) =12.8

Identification
A major single band of approx. 220,000 Dalton is evident.

Suggested coating procedure
The Fibronectin should be added to the growth medium in the culture vessel which is then placed in an incubator 30-60 minutes before seeding. The recommended concentration of the Fibronectin is 5 micrograms per ml of medium. When the medium is replaced in the days following initial seeding, no further Fibronectin is required.

1. Ruoslahti E.
   Int. J. Cancer 20, 1-15 (1977)
2. Miekka S.I Et Al
   Thrombosis Research 27, 1-14 (1987)
3. Mosesson M.W
   The J. of Biological Chemistry
   Vol.245, No.21 5728-5736 (1970)





Collagen Type I, Rat Tail

Product Name	Catalogue No.	Unit Size	Storage Temp.
Collagen Type I, Rat Tail	41-843-25	25mg	2-8ºC
	41-843-100	100mg	2-8ºC

Collagens are a family of highly characteristic fibrous protein found in all multicellular animals and are critical in cell adhesion. Collagen Type I is found in several tissues including skin, connective tissue cartilage and bone. Collagen Type I is an attachment factor that facilitates the attachment and cytoplasmic spreading of all types of anchorage-dependent cells, when used as a thin layer on a tissue culture surface. As a gel, Collagen I enhances expression of cell-specific morphology and function.

Cell Qualified 0.1% Gelatin Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Gelatin Solution (0.1%)	01-944-1A	500ml	2-8ºC
	01-944-1B	100ml	2-8ºC

Qualified for Mouse Embryonic Stem (ES) Cells

Mouse Embryonic Stem Cells (MESCs) are used to generate mouse mutants by gene targeting and blastocyst-mediated transgenesis. Undifferentiated ES cells may be maintained in vitro for extended periods without loss of differentiation capacity when re-implanted back into a blastocyst. Well-established general culture conditions usually require the undifferentiated ES cells to be grown on inactive feeder cell layers or on gelatin-coated plates with Leukemia Inhibitory Factor (LIF) in the culture medium to influence cell growth and function. Growth and differentiation of anchorage-dependent cells are strongly influenced by glass or plastic cultureware offered as a cell-substrate interactive platform. Cell growth rates may be exponentially improved by specialized surface treatments or coating with attachment factors such as Gelatin Solution with LIF.

Application
Used for the attachment of a variety of cell types.

Properties
Sterile, Endotoxin Tested and Cell Culture Tested.
Product is ready to use for plating.

Nutrimatrix™ - ECM Coated Plastic Ware

Coated with extracellular matrix (ECM) simulates in vivo conditions

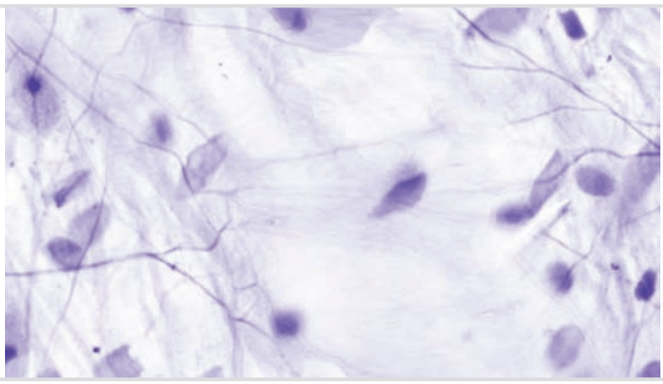
One of the drawbacks in growing cells In Vitro using conventional tissue culture techniques is that the cells rest on plastic rather than on their natural biological support. This natural support is a complex network of macromolecules known as the extracellular matrix or ECM. ECM holds cells and tissues together and provides a highly organized lattice within which cells can migrate and interact with each other. The matrix plays an active and complex role in regulating the behavior of cells that are in contact with it, influencing their shape, migration, proliferation and metabolic functions. In contrast, cells grown on plastic lose many of their natural differentiated properties due to the lack of interaction with ECM.

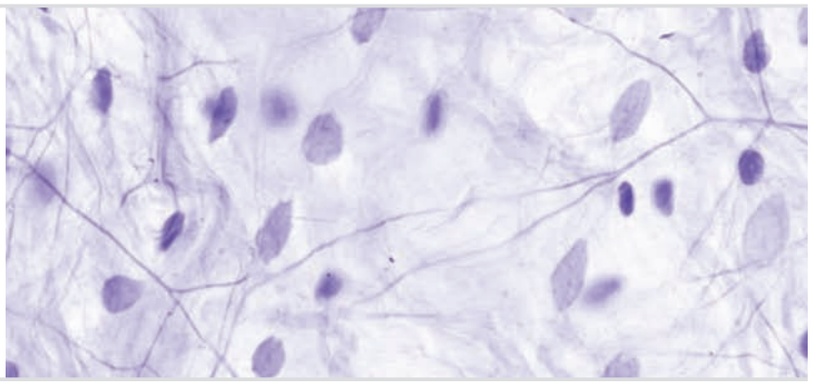
ECM is composed of different types of collagen glycosaminoglycans, proteoglycans and glycoproteines[1]. It resembles the vascular subendothelial basal lamina in its organization and macromolecular constituents (fibronectin, lamin, collagen types III, IV and V, and sulfated proteoglycans)[2].

Advantages
Rapid attachment; high plating and cloning efficiencies; rapid proliferation, high saturation density; lower requirements for serum and added growth factors; better response to physiologically occurring hormones; expression of differentiated functions; longer life span for cells; flattening and morphological changes; and improved plating consistency.

Among the cells types showing a favorable response to ECM are human, bovine and other origin.

Research Applications
- Epithelial Cells:
  Nutrimatrix™ ECM-coated plastic vessels with serum-free medium enable a higher rate of success in growing normal and malignant human epithelial cells from biopsy specimens. ECM induces changes in cell shape not observed in cells grown on plastic or isolated components of the ECM. Cells which for different reasons do not flatten or spread on plastic do so rapidly on ECM.
- Hormone Secretion Research:
  Nutrimatrix™ ECM-coated plastic vessels with serum-free media support the maintenance and normal function of hormone secreting cells such as pancreatic islet cells, hepatocytes pituitary cells, granulosa cells, etc.





- Secretion of Cellular Products:
  The ECM/serum-free medium combination promotes research possibilities on various cellular products due to the following multiple effects:
- Hormone Response Research:
  ECM effects cell shape and hormone responsiveness. As expected the cells do not respond when maintained on artificial substrata or isolated components of the ECM.

Biotechnology Applications[3]

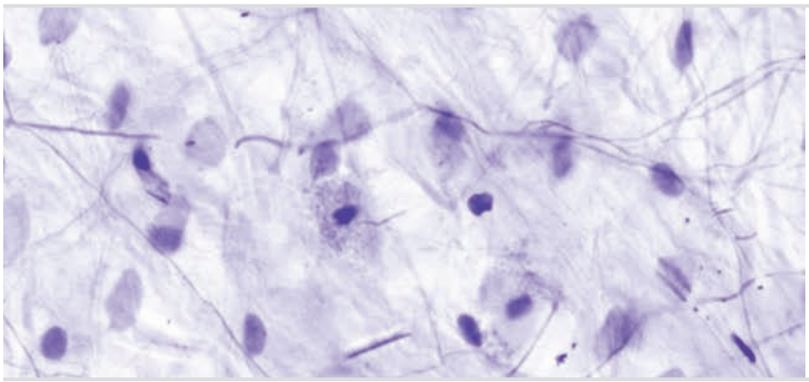
- Yield and Differentiation:
  The maintenance and growth of differentiated cells on ECM is expected to promote a high yield of various hormones and growth factors in tissue culture.
- Purification:
  Growth of cells in serum-free media will facilitate the purification of various cellular products that are secreted into the medium. Purification will be relatively simple due to the absence of serum proteins.
- Production:
  Large-scale growth of cells on ECM can be performed in bulk cell culture Nutrimatrix™ vessels coated with ECM, or on Nutrimatrix™ ECM-coated microcarriers. Using these techniques, continuous rather than batch processes can be developed.
- Growth Factor Secretion:
  Growth factors may be produced in better yields by human cells cultured on ECM rather than on plastic and can then be purified and used for research and clinical applications.
- In Vitro Toxicological Testing And Drug Screening:
  The growth of cells on ECM in serum free medium may reduce the cost and simplify the procedure of studying the effect on cells of single drugs, drug combinations and hormones or where a single component is being tested at a time.
- Neurobiology:
  ECM has been shown to support the attachment and maintenance of neurons from various sources and to promote the outgrowth and directed elongation of neurites.

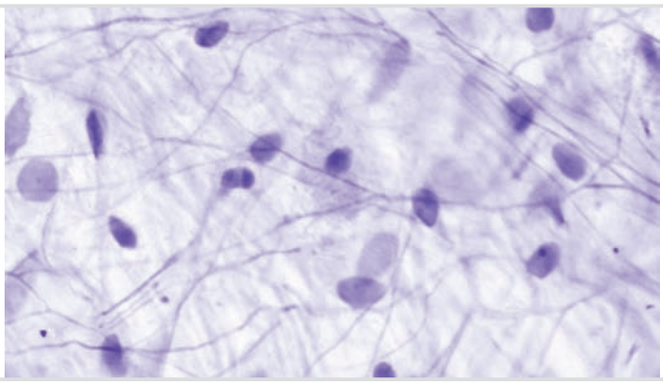
Item	Packaging (unit/Pack)	Catalogue No.
Tissue Culture Dishes 35 mm	5	E-TCP-35
Tissue Culture Dishes 60 mm	5	E-TCP-60
Tissue Culture Dishes 90 mm	5	E-TCP-90
Tissue Culture Flasks 25 cm^2	5	E-TCF-25
Tissue Culture Flasks 80 cm^2	5	E-TCF-80
Microtiter 96-Well Plate	1	E-TCMT-F
4-Well Culture Plate	1	E-TCMW-4
6-Well Culture Plate	1	E-TCMW-6
12-Well Culture Plate	1	E-TCMW-12
24-Well Culture Plate	1	E-TCMW-24
Coverslips (Round, 22 mm)	5	E-TCCS-P22
Four 13mm Coverslips In 4-Well Plate	1	E-TC-IF-13
Eight 12mm Filters in 24- Well Plate	1	E-TC-M-12
Eight Well Lab-Tek Chamber Slide	1	E-LT-8

Storage

Nutrimatrix™ ECM-coated plastic vessels are shipped at ambient temperature and should be stored at 2-8ºC upon arrival.

(1) Cancer research 46: 3653 (1986)
(2) Blood 65: 1477 (1985)
(3) Metal Ions in Biology and Medicine. Volume 4 by Philippe Collery. Published by John Libbey Eurotext, 1996






BIOLOGICAL INDUSTRIES

BALANCED SALT SOLUTIONS

08

BALANCED SALT SOLUTIONS

Balanced Salt Solutions, for all intents and purposes, are inorganic salt solutions that form the basis of many complex media formulations. They may contain varying amounts of NaCl, KCl, $MgCl_2$, $NaHCO_3$, $MgSO_4$, $CaCl_2$ and other salts, and have since been modified and enriched along with amino acids, vitamins, fatty acids and lipids and as well as other nutrients that segue into a final medium based upon application and technique to meet the cells unique niche requirements. These precise media formulations are now optimized to the n^{th} degree to support a wide array of cell lines. The current role of a balanced salt solution in cell culture is multi-faceted and may be divided into four principal functions.

- Functions as a diluent, as an irrigating medium or transporting fluid while maintaining osmoregulation, the optimal and constant balance of osmotic pressure gradients between the intracellular and extracellular compartments.
- Provides cells with fluids and certain bulk inorganic ions essential for normal cell metabolism.
- When combined with a carbohydrate, such as Glucose ($C_6H_{12}O_6$), it provides a primary energy source for cell metabolism.
- When provided with a buffering system, it facilitates the maintenance of physiological pH within an acceptable range of 7.1-7.5.

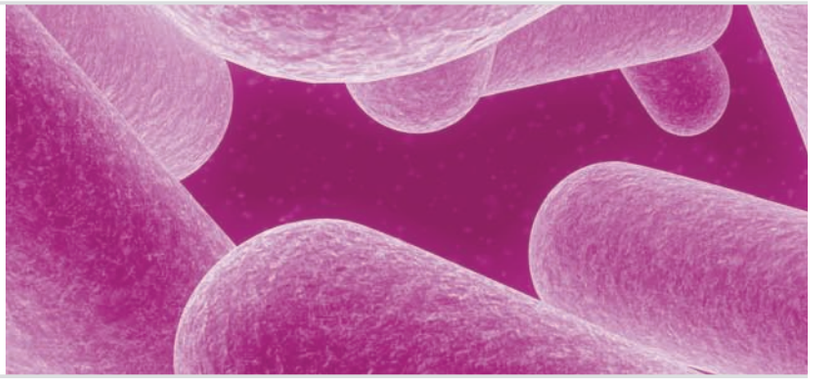
Biological Industries offers a wide range of various formulations to meet all the requirements for cell culture. Each batch undergoes extensive and vigorous Quality Control Protocols to verify compliance with product specifications. Each batch undergoes a series of Chemical, Microbiological, Stability, and Performance Testing.

Product Name	Concentration	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Gey's Balanced Salt Solution	1X	01-919-1A	500ml	AMB	
Earle's Balanced Salt Solution	1X	02-010-1A	500ml	AMB	135
		02-010-1B	100ml	AMB	135
Earle's Balanced Salt Solution Without Sodium Bicarbonate	10X	02-010-5A	500ml	AMB	135
		02-010-5B	100ml	AMB	135
Earle's Balanced Salt Solution Without Phenol Red	1X	02-011-1A	500ml	AMB	
		02-011-1B	100ml	AMB	
Earle's Balanced Salt Solution Without Phenol Red Without Sodium Bicarbonate	10X	02-011-5A	500ml	AMB	
		02-011-5B	100ml	AMB	
Hanks' Balanced Salt Solution	1X	02-015-1A	500ml	AMB	135
		02-015-1B	100ml	AMB	135
Hanks' Balanced Salt Solution Without Sodium Bicarbonate	10X	02-015-5A	500ml	AMB	135
		02-015-5B	100ml	AMB	135
Hanks' Balanced Salt Solution Without Phenol Red	1X	02-016-1A	500ml	AMB	
		02-016-1B	100ml	AMB	
Hanks' Balanced Salt Solution Without Calcium and Magnesium	1X	02-017-1A	500ml	AMB	
		02-017-1B	100ml	AMB	
Hanks' Balanced Salt Solution Without Calcium and Magnesium Without Phenol Red	1X	02-018-1A	500ml	AMB	
		02-018-1B	100ml	AMB	
Dulbecco's Phosphate Buffered Saline (DPBS)	1X	02-020-1A	500ml	2-8ºC	135
		02-020-1B	100ml	2-8ºC	135
Dulbecco's Phosphate Buffered Saline (DPBS) Without Calcium and Magnesium	1X	02-023-1A	500ml	AMB	135
		02-023-1B	100ml	AMB	135
Dulbecco's Phosphate Buffered Saline (DPBS) Without Calcium and Magnesium	10X	02-023-5A	500ml	AMB	135
		02-023-5B	100ml	AMB	135
Spinner Modified Salt Solution	1X	02-030-1A	500ml	AMB	135
Alsever's Solution	1X	02-045-1A	500ml	AMB	135
		02-045-1B	100ml	AMB	135

ANTIBIOTICS

09

ANTIBIOTICS



Antibiotic / Antimycotic Solutions

Antibiotics are natural substances of bacterial origin derived entirely or partially from certain microorganisms that are used to treat bacterial or fungal infections by selective inhibition. Chemotherapeutic agents refer to any synthetic or man-made substance that actually characterizes the so-called newer antibiotics today that are essentially chemically-modified or chemically synthesized biological products. Today, the term 'antibiotic' is used to refer to all types of antimicrobial agents. The distinctions between both natural and man-made synthetic substances are designed, in one way or another, to block one or several crucial metabolic pathways without untoward manifestations to the host, or in this case, the cell culture. Preventing cell culture contamination is an essential part of all animal cell culture. The risk of contamination may be eliminated by effective aseptic/sterile techniques and the judicious use of antibiotics.

Antibiotics may be classified into several key groups by virtue of their mechanism of action which include:
- Inhibition of Cell-Wall Synthesis
- Inhibition of Nucleic Acid Synthesis (i.e. RNA/DNA)
- Inhibition of Protein Synthesis
- Inhibition or Interference of Microtubule Function

The major advantage of some antibiotics is their ability to selectively target crucial and specific cell processes which either kill the microorganism in question or prevent them from reproducing unabated. Antibiotics are often also categorized by:
- Their Spectrum of Activity
- Their Bacteriostatic/Bactericidal Properties
- Their Gram-Negative or Gram-Positive Characteristics

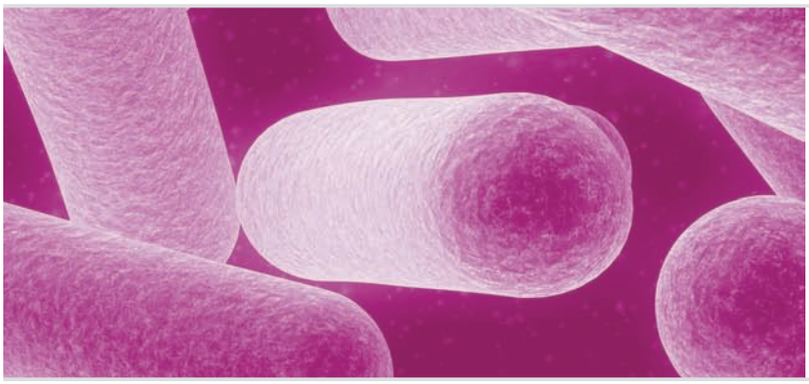
Antibiotics are ineffective against viruses.

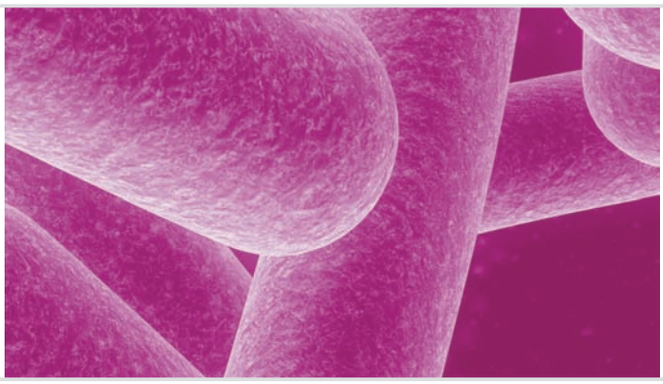
Although many laboratories use antibiotics on a regular basis, the decision to use them to prevent cell culture contamination must be based on the individual researcher's requirements and experience.

The appropriate antibiotics may be added to culture media to eliminate microbial contaminants. The most commonly encountered microorganisms are bacteria, yeast, other fungi and mycoplasma while the most common routes of contamination are poor aseptic technique and use of non-sterile medium components.

Biological Industries offers a wide range of effective antibiotics that include solutions, mixtures, and powdered chemical formulations. The following table is presented as a general guide for use in cell culture.

Product Name	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Amphotericin B[2] Solution 250 microgram/ml	03-028-1B	100ml	-20ºC	136
	03-028-1C	20ml	-20ºC	136
Amphotericin B[2] Solution 2500 microgram/ml	03-029-1B	100ml	-20ºC	136
	03-029-1C	20ml	-20ºC	136
Nystatin[1] Suspension 10,000 units/ml	03-030-1C	20ml	-20ºC	136
Penicillin-Streptomycin Solution Penicillin G Sodium Salt, 10,000 units/ml Streptomycin Sulfate, 10mg/ml	03-031-1B	100ml	-20ºC	136
	03-031-1C	20ml	-20ºC	136
Penicillin-Streptomycin Solution, 10X Conc. Penicillin G Sodium Salt , 100,000 units/ml Streptomycin Sulfate, 100mg/ml	03-031-5B	100ml	-20ºC	136
	03-031-5C	20ml	-20ºC	136
Penicillin-Streptomycin Nystatin[1] Solution Penicillin G Sodium Salt, 10,000 units/ml Streptomycin Sulfate, 10mg/ml Nystatin[1], 1,250 units/ml	03-032-1B	100ml	-20ºC	136
	03-032-1C	20ml	-20ºC	136
Penicillin-Streptomycin Amphotericin B[2] Solution Penicillin G Sodium Salt, 10,000 units/ml Streptomycin Sulfate, 10mg/ml Amphotericin B[2], 25 microgram/ml	03-033-1B	100ml	-20ºC	136
	03-033-1C	20ml	-20ºC	136
Penicillin-Streptomycin Neomycin Solution Penicillin G Sodium Salt, 10,000 units/ml Streptomycin Sulfate, 10mg/ml Neomycin Sulfate, 10mg/ml	03-034-1B	100ml	-20ºC	136
	03-034-1C	20ml	-20ºC	136





Product Name	Catalogue No.	Unit Size	Storage Temp.	Formulation Page
Gentamicin Sulfate Solution 50 mg/ml	03-035-1B	100ml	AMB	136
	03-035-1C	20ml	AMB	136
Kanamycin Sulphate Solution 10mg/ml	03-049-1B	100ml	-20ºC	136
	03-049-1C	20ml	-20ºC	136

(1) **Nystatin** is the generic name for **Mycostatin®** which is the registered trade mark of E.R. Squibb & Sons.
(2) **Amphotericin B** is the generic name for **Fungizone®** which is the registered trade mark of E.R. Squibb & Sons.

Use of Antibiotics in Mammalian Cell Culture

Antibiotics are secondary metabolites which are produced by certain strains of bacteria and fungi. In cell culture, antibiotics have long been used to prevent the growth of contaminating bacteria and fungi.
The following table is provided as a guide for antibiotics selection and appropriate concentrations. Refer to pharmacology guides for antibiotic incompatibilities and other properties not included in the table.

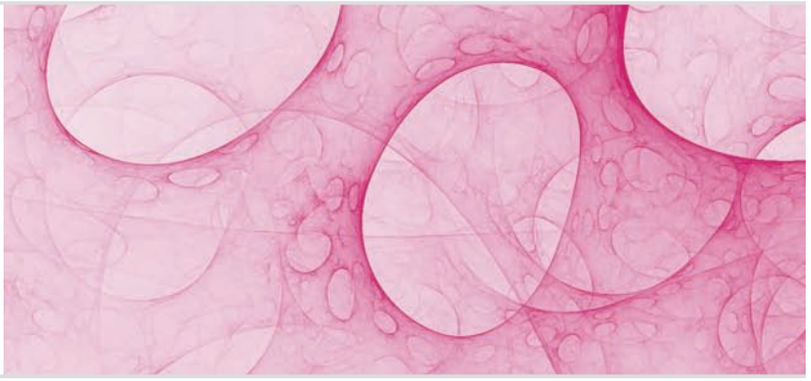
Product Name	Catalogue No.	Concentration	Storage Temp.	Mode of Action	Suggested Working Conc.
Amphotericin B	03-028-1	250µg/ml	-20ºC	Inhibition of cell membrane permeability (fungi and yeasts)	1-10ml/lit
Amphotericin B	03-029-1	2,500µg/ml	-20ºC	Inhibition of cell membrane permeability (fungi and yeasts)	0.1-1ml/lit
Nystatin*	03-030-1	10,000un/ml	-20ºC	Inhibition of cell membrane permeability (fungi and yeasts)	1-10ml/lit
Penicillin-Streptomycin	03-031-1	Penicillin: 10,000un/ml Streptomycin: 10mg/ml	-20ºC	Penicillin: Inhibition of cell wall synthesis Streptomycin: Inhibition of protein synthesis by binding to 30S subunit of the bacterial ribosome	10ml/lit
Penicillin-Streptomycin 10x	03-031-5	Penicillin: 100,000un/ml Streptomycin: 100mg/ml	-20ºC	See: Penicillin-Streptomycin	1ml/lit
Penicillin-Streptomycin-Nystatin	03-032-1	Penicillin: 10,000un/ml Streptomycin: 10mg/ml Nystatin: 1,250un/ml	-20ºC	See: Penicillin, Streptomycin and Nystatin	10ml/lit
Penicillin-Streptomycin-Amphotericin B	03-033-1	Penicillin: 10,000un/ml Streptomycin: 10mg/ml Ampho. B: 25µg/ml	-20ºC	See: Penicillin, Streptomycin and Amphotericin B	10ml/lit
Penicillin-Streptomycin-Neomycin	03-034-1	Penicillin: 10,000un/ml Streptomycin: 10mg/ml Neomycin: 10mg/ml	-20ºC	See: Penicillin and Streptomycin Neomycin: Inhibition of protein synthesis by binding to 30S subunit of the bacterial ribosome	10ml/lit
Gentamicin sulfate	03-035-1	50mg/ml	15-30ºC	Inhibition of protein synthesis by binding to 30S subunit of the bacterial ribosome	1ml/lit
Kanamycin sulfate	03-049-1	10mg/ml	-20ºC	Inhibition of protein synthesis by binding to 30S subunit of the bacterial ribosome	10ml/lit

* Suspension in water

CELL DISSOCIATION PRODUCTS

10

CELL DISSOCIATION PRODUCTS



Most cell cultures grow as a single thickness cell layer or sheet attached to a substrate known as a monolayer. When subculturing adherent cells, these intercellular and cell-to-substrate links or connections must be gently dissociated. Proteolytic enzymes, such as trypsin (i.e. a serine peptidase), breaks or gently separates these bonds by creating a single-cell suspension from which new subcultures are realized. Trypsin solutions are widely utilized as cell dissociation reagents for continuous cell culture of adherent growing cells. Trypsin proteolysis or trypsinization is a process in which proteins have been digested or treated with trypsin and are thus said to be trypsinized. Biological Industries' Trypsin is designed to gently dissociate cells from almost any support substrates, as well as from each other, in order to actualize cell manipulation techniques, and for other studies that require intact cell-surface proteins. Trypsin is available in a varied array of formulations with or without EDTA. EDTA is a chelator that binds calcium and magnesium ions that may otherwise inhibit the trypsin activity, which then hydrolyzes and gains access to the intercellular bonds (cell-cell and/or cell-substrate bonds).

Crystalline Trypsin Solution & Soybean Trypsin Inhibitor Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Crystalline Trypsin Solution (0.02%) Without Phenol Red	03-047-1A	500ml	-20ºC
	03-047-1B	100ml	-20ºC
Soybean Trypsin Inhibitor 50X Conc., 5mg/ml	03-048-1C	20ml	-20ºC

Crude trypsin is often the subculturing agent of choice for cell dissociation/disaggregation of adherent cells, although the treatment may be cytotoxic if prolonged. Over-trypsinization is a common cause of subculture problems. Regarding the use of crude trypsin, some important facts must be noted:

- Cells must **NEVER** remain in the crude trypsin for longer than 3-5 minutes as they may be seriously damaged in the process (i.e. damage to the intracellular proteins).
- Cells should **NEVER** be left without a fluid layer.

The use of crystalline trypsin, rather than crude trypsin, most often performs better long-term cell growth in serum-free medium formulations. It is specifically formulated to have a gentle nature with much better cell viability, in which the cells are not subject to the vagaries of time and circumstance as when the cruder forms of trypsin are utilized.

Some of the advantages of crystalline trypsin versus the cruder trypsin forms:

1. Crystalline trypsin **does not** damage cells after prolonged exposure.
2. Crystalline trypsin **does not** require multiple-change procedures and thus is less labor-intensive.
3. Crystalline trypsin maintains better cell viability and enhances the process of cell passaging.
4. Crystalline trypsin is not as cytotoxic to cells with all the negative ramifications of crude trypsin.
5. Biological Industries' Crystalline Trypsin Solution also contains additives that protect the cell wall, enhancing cell viability.

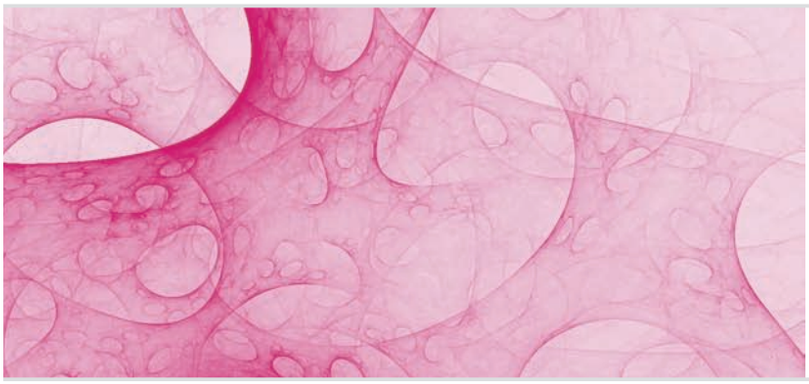
In a serum-free culture environment, the cells must be separated by rapid centrifugation or by utilizing trypsin inhibitors such as Soybean Trypsin Inhibitor (SBTI). SBTI is a single polypeptide that forms a stable, stoichiometric, enzymically inactive complex with trypsin, thereby reducing the availability of trypsin by somewhat binding chymotrypsin. With Biological Industries' Soybean Trypsin Inhibitor Solution, any excess Crystalline Trypsin Solution may be completely neutralized, thereby avoiding the use of serum for this purpose. The cells may then be re-suspended successfully in a suitable growth medium.

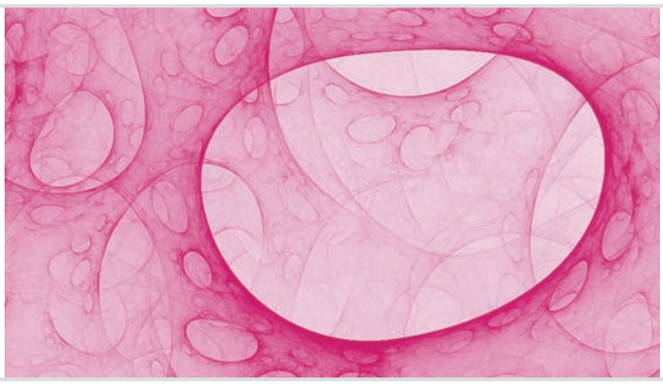
The use of animal-derived components in Biopharmaceutical Manufacturing is experiencing ever-increasing regulatory scrutiny. Therefore, there is the need to develop non-animal source products for cell culture. Trypsin is an essential product for cell culture manipulation. However, it is purified from animal-source materials with one unfortunate notable disadvantage: contamination from variegated sources such as viruses, other potential adventitious agents and other unwanted enzymes.

Non-Enzymatic Cell Dissociation Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Non-Enzymatic Cell Dissociation Solution	03-071-1B	100ml	2-8ºC

Cell Dissociation Solution is a special, non-enzymatic formulation with a proprietary mixture of chelators for gently dislodging adherent cell types from culture vessels. Cell Dissociation Solution helps to maximize the yield of functionally viable cells from these culture vessels. It is a non-enzymatic, protein-free and animal-component free solution. Another major advantage is that cells can be exposed to this solution for longer periods of time without the risk of subjecting them to protein digestive enzymes such as trypsin. However, the solution is not recommended for cells with very adhesive properties. For those cell lines which are difficult to dislodge, Biological Industries has developed a Papain Dissociation Solution.





Features

Contains a proprietary mixture of chelators. Contains no enzymes or proteases.

- Works with serum-free and serum-containing media.
- Reduces the risk of cell damage associated with trypsin.
- Chemically defined.
- Contains no products of animal origin.
- Supplied as a ready-to-use solution.

Papain Dissociation Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Papain Dissociation Solution	03-072-1B	100ml	-20ºC

Papain is a nonspecific, endolytic, sulfhydrl protease or protein-cleaving enzyme, known as cysteine-endopeptidase, and is derived and isolated from papaya fruit (i.e. Carica papaya). More specifically, it is isolated from the papaya latex, which is then utilized in a wide variety of applications. Papain is commonly used in cell isolation procedures, where it has proven to be more efficient and less destructive than other proteases on certain tissues such as and including, among others, the dissociation of retinal neurons[1], in the preparation of primary neurons from the visual cortex of postnatal rats[2], and for the isolation of smooth muscle cells[3].

Papain has a wide specificity in that it will degrade most protein substrates more extensively than the pancreatic proteases and has been proven not only to manifest fewer untoward and negative ramifications producing less cell and tissue trauma, but also to be much more effective than other available proteases. Biological Industries' Papain Dissociation Solution is a ready-to-use solution and is one of our non-animal alternatives for trypsin.

Physical Properties and Kinetics

Papain is a cysteine protease hydolase enzyme of the peptidase C1 family derived from the papaya family, Carica papaya and the mountain papaya, Vasconcellea cundinamarcensis. It consists of a single peptide chain with three disulfide bridges and a sulfhydrl group necessary for the activity of the enzyme.

Specificity

Papain is more effective in digesting most protein substrates more extensively and effectively than pancreatic proteases. It further exhibits broad specificity cleaving peptide bonds of such basic amino acids as leucine and glycine. In addition to the aforementioned activity, it also hydrolyzes esters and amides.

(1) Shen J., et al., Japanese Journal of Physiology,1995

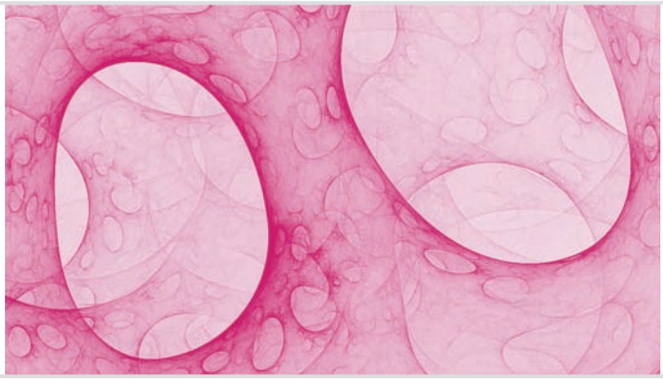
(2) Huettner, J.E. Baughman, R.W., Journal Of Neuroscience, 1986

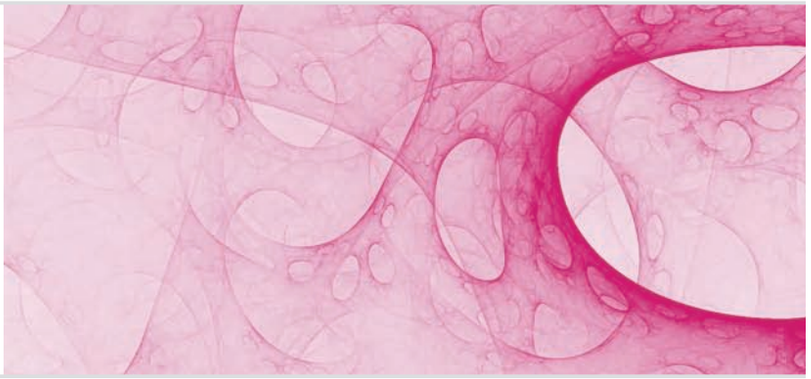
(3) Kinoshita, K. et.al., American Journal of Physiology, Gastrointestinal and Liver Physiology, 2003 and Driska,S.P. et.al., Journal of Applied Physiology, 1999.

Accutase Solution, primary human cell culture tested

Product Name	Catalogue No.	Unit Size	Storage Temp.
Accutase Solution, primary human cell culture tested	03-073-1B	100ml	-20ºC

Accutase is an alternative cell detachment solution to trypsin and can also be used for tissue dissociation. It is a ready to use solution and was developed for very gentle and effective detachment of adherent cells. The well balanced combination of protease and collagenolytic activities ensures that surface proteins and epitopes stay entirely intact. This makes it perfectly suited for applications, which require unchanged surface conditions.





Product Name	Trypsin Concentration	Catalogue No.	Unit Size	Storage Temp.
Trypsin Solution A With Calcium and Magnesium Without Phenol Red	0.25%	03-045-1B	100ml	2-8ºC
Trypsin Solution B Without Calcium and Magnesium Without Phenol Red	0.25%	03-046-1A	500ml	-20ºC
		03-046-1B	100ml	-20ºC
Trypsin Solution B Without Calcium and Magnesium Without Phenol Red 10x Concentrate	2.50%	03-046-5A	500ml	-20ºC
		03-046-5B	100ml	-20ºC
Crystalline Trypsin Solution Without Phenol Red	0.02%	03-047-1A	500ml	-20ºC
		03-047-1B	100ml	-20ºC
Soybean Trypsin Inhibitor **50x Conc., 5mg/Ml**		03-048-1C	20ml	-20ºC
Trypsin EDTA Solution A **EDTA (0.02%)** With Phenol Red	0.25%	03-050-1A	500ml	-20ºC
		03-050-1B	100ml	-20ºC
Trypsin EDTA , EDTA 0.2% , 10X Conc.*	0.50%	03-051-5B	100ml	-20ºC
		03-051-5C	20ml	-20ºC
Trypsin EDTA Solution B **EDTA (0.05%)** With Phenol Red	0.25%	03-052-1A	500ml	-20ºC
		03-052-1B	100ml	-20ºC
Trypsin EDTA Solution C **EDTA (0.02%)** With Phenol Red	0.05%	03-053-1A	500ml	-20ºC
		03-053-1B	100ml	-20ºC
Trypsin EDTA Solution C **EDTA (0.02%)** Without Phenol Red	0.05%	03-054-1A	500ml	-20ºC
		03-054-1B	100ml	-20ºC
Non-Enzymatic Cell Dissociation Solution		03-071-1B	100ml	2-8ºC
Papain Dissociation Solution		03-072-1B	100ml	-20ºC
Accutase Solution, primary human cell culture tested		03-073-1B	100ml	-20ºC

* See Chapter 11- Cytogenetics

See formulations on page 139


BIOLOGICAL INDUSTRIES
KIBBUTZ BEIT HAEMEK ISRAEL
STERILE CE
TRYPSIN INHIBITOR
SOLUTION (From soybean)
CONCENTRATE x 50
Store at -20°C
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Exp. 10/2009
Vol. 100ml
Lot: 818682
For In Vitro Diagnostic Use
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CRYSTALLINE TRYPSIN
SOLUTION
200 mg/l
BIOLOGICAL INDUSTRIES
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STERILE CE
CELL DISSOCIATION
SOLUTION
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Animal component free
Cat. 03-071-1B
Store:+2 to 8°C.
Exp: 05/2011
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HUMAN CYTOGENETICS

Prenatal Diagnostics
Peripheral Blood
Bone Marrow
Auxiliary Products

11

HUMAN CYTOGENETICS

Prenatal Diagnostics

Optimized Media for Culture and Genetic Analysis of Human Amniotic Fluid Cells and Chorionic Villi (CV) Samples

Chromosome Karyotyping was first developed in the field of Cytogenetics. The basic principle of the method is the preparation of chromosomes for microscopic observation by arresting cell mitosis at metaphase with colchicine and treating the cells with a hypotonic solution. This is followed by regular or fluorescent staining of the chromosomes, which are then tested with the aid of a microscope and computer programs to arrange and identify the chromosomes for the presence of genetic abnormalities.
In principle, this method enables the identification of any abnormality - excess chromosomes or chromosome deficiency, broken chromosomes, or excess genetic material (as a result of a recombination process).
Clinical cytogenetics laboratories use this method with amniotic fluid, chorionic villi, blood cells, skin cells, and so on, which can be cell cultured to obtain mitotic cells.
Most amniotic fluid cells originate from the fetus and include fibroblasts, epithelial cells and amniocytes. The cells suited for genetic analysis are fibroblasts and amniocytes, and chromosome preparation from these cells yields a clear picture of the chromosomes for microscopic observation.
Amniocentesis is typically carried out in week 16-20 of pregnancy, when 20-40ml of amniotic fluid is drawn for genetic analysis.
The cells can be seeded on a slide or in suitable flasks to obtain colonies or cell cultures. Since the cells divide, chromosome karyotyping can be carried out on them for general genetic testing. To test specific abnormalities, a small number of cells can be taken from the original sample for FISH and/or QF-PCR testing.
The time that elapses until the final results of genetic analysis are obtained is of significant importance both from an emotional point of view – the tension and stress entailed in waiting for the final results - and a practical one - the need to terminate pregnancy if genetic abnormalities are found. Pregnancy termination in the second trimester in effect means performing an abortion; hence the importance of obtaining results as early as possible in order to alleviate the procedure.
In the past decade, Biological Industries Ltd. has developed a range of cytogenetics products, including media for culture of amniotic fluid and chorionic villi cells, BIOAMF-1, BIOAMF-2 and BIOAMF-3, which are selling very successfully throughout the world.

BIOAMF-1 Basal Medium and Supplement

Product Name	Catalogue No.	Unit Size
BIOAMF-1 Basal Medium	01-190-1A	450ml
	01-190-1B	90ml
BIOAMF-1 Supplement	01-192-1D	10ml
	01-192-1E	50ml

BIOAMF-1 is designed for the primary culture of human amniotic fluid cells and chorionic villi (CV) samples in both open (5% CO_2) and closed systems. The medium allows rapid growth of amniocytes or chorionic villi for use in karyotyping.
No supplementation with serum or serum-substitutes is necessary.
The medium consists of two components: basal medium and frozen supplements.

Instructions for Use
For the preparation of 500ml complete medium, use 01-190-1A with 01-192-1E. For the preparation of 100ml complete medium, use 01-190-1B with 01-192-1D. Thaw the BIOAMF-1 Supplement by swirling in a 37ºC water bath, and transfer the contents to the bottle of BIOAMF-1 Basal Medium. Mix the complete medium by swirling the bottle, and add 2mM L-Glutamine (L-Glutamine Solution 200mM, cat. no. 03-020-1). Antibiotics may be added if desired (Pen-Strep, cat. no. 03-031-1).

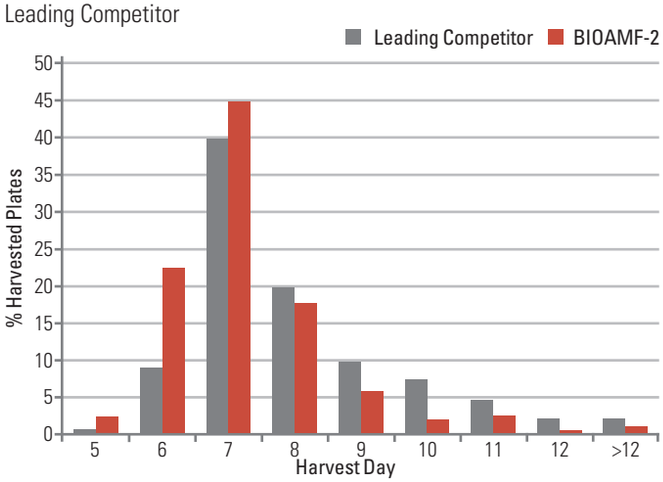
Storage and Stability
BIOAMF-1 Basal Medium is stable for 24 months from production date when stored at 2-8ºC.
BIOAMF-1 Supplement is stable for 15 months from production date when stored at -20ºC.
The complete medium is stable for 7 days when stored at 2-8ºC.
Do not freeze the complete medium. Protect both the basal medium and the complete medium from light.

BIOAMF-2 Complete Medium

Product Name	Catalogue No.	Unit Size
BIOAMF-2 Complete Medium	01-194-1A	500ml
	01-194-1B	100ml

BIOAMF-2 is a complete medium specifically optimized for the primary culture of human amniotic fluid cells and chorionic villi (CV) samples in both open (5% CO_2) and closed systems.
No addition of serum is required, and chromosome karyotyping time is greatly reduced compared with the conventional medium.
Note: this is a one-bottle formulation, which also contains L-Glutamine and antibiotics. Simply thaw and use!

Figure 1: Comparison of the Percentage of Harvested Plates According To Harvest Day Between BIOAMF-2 By Biological Industries and A Leading Competitor



Storage and Stability
BIOAMF-2 Medium should be kept frozen at -20°C. After thawing, the medium should be stored at 2-8°C. The medium should be used within 7 days after thawing. Protect the medium from light.

BIOAMF-3 Complete Medium

Product Name	Catalogue No.	Unit Size
BIOAMF-3 Complete Medium	01-196-1A	500ml
	01-196-1B	100ml

An improved version of complete medium specifically optimized for the primary culture of human amniotic fluid cells and chorionic villi samples used in prenatal diagnostic testing. This medium accelerates the growth of the non-epithelial cells used for chromosome karyotyping.
The medium is supplied frozen and contains L-Glutamine and antibiotics.

Advantages of BIOAMF-3
- Enhanced buffering capacity both in open (CO_2) and closed systems.
- Improved banding quality: excellent chromosome morphology and metaphase structure.
- Increased metaphase yield.
- Extended stability of the medium at 2-8°C.

Storage and Stability
BIOAMF-3 Medium should be kept frozen at -20°C. After thawing, the medium should be stored at 2-8°C. The medium should be used within 14 days after thawing. Protect the medium from light.

Blood Lymphocyte Culture

Blood cell karyotyping is an important tool in modern human cytogenetics, providing information about chromosomal abnormalities, their frequency in the population, and the relationship between specific chromosomal abnormalities and phenotypic effects.
Human cytogenetic studies involve the examination of a stimulated lymphocyte after blocking cell division at metaphase with an inhibitor of spindle formation. The nuclear membrane breaks down and chromosome condensation takes place as usual, but the chromosomes fail to organize themselves into a metaphase plate.
This gives an appearance quite unlike a natural metaphase, in that the chromosomes are free within the cytoplasm. Subsequent processing and staining allows clear visualization of the chromosomes.
The chromosomes can be stained either by a technique that gives a fairly uniform intensity, or by a technique that gives differential staining along the length of the chromosome.

Biological Industries offers a ready-to-use flat bottom tube which contains 5ml of a complete medium for Peripheral Blood Karyotyping. The medium contains PHA. A slated rack is supplied with each 10 tubes so that the tubes are put into the incubator at the right angle.

Advantages
- Saves time
- Excellent growth promotion
- No other supplements required
- Slanted rack for convenient incubation

Peripheral Blood Karyotyping Medium

Product Name	Catalogue No.	Unit Size
Peripheral Blood Karyotyping Medium Without Phytohemagglutinin	01-198-1A	500ml
	01-198-1B	100ml
Peripheral Blood Karyotyping Medium With Phytohemagglutinin	01-201-1A	500ml
	01-201-1B	100ml
	01-201-1H	5ml

Peripheral Blood (PB) Karyotyping Medium is specifically optimized for short-term culture of peripheral blood lymphocytes for chromosome analysis. No addition of serum, glutamine or antibiotics is required.The medium is supplied frozen.

Storage and Stability
PB Karyotyping Medium should be kept frozen at -20ºC. After thawing, the medium should be stored at 2-8ºC. The medium should be used within 10 days after thawing. Protect the medium from light.

Bone Marrow Culture

Cytogenetic analysis of human hematopoietic cells using bone marrow aspirates is a standard practice in hematology. Cell culture improvements and processing techniques have enabled the identification of a number of recurring abnormalities in solid tumors and hematologic malignant diseases. But even more data are available for leukemias and lymphomas than for solid tumors because of the relative ease of obtaining bone marrow or peripheral blood specimens from leukemia patients.
The study of chromosomal abnormalities in leukemia serves two functions: The first is to assist in more accurate diagnosis, thereby providing prognostic information and allowing the more rational selection of therapy for a particular patient. The second is to identify the sites of consistent rearrangements, providing the precise localization required for the isolation and cloning of DNA from these regions. Using molecular techniques the function of the genes can be identified and the mechanisms whereby their altered function is involved in tumorigenesis can be determined.
In the past, it was assumed that cytogenetic analysis of hematologic malignant disorders was best performed directly on uncultured bone marrow samples. However, later studies indicate that analysis of cultured samples disclosed a clonal abnormality that would not have been detected if the direct method alone had been used. Thus, for many samples, chromosomal rearrangements were often characterized only after analysis of cultured preparations.

Bone Marrow Karyotyping Medium

Product Name	Catalogue No.	Unit Size
Bone Marrow Karyotyping Medium Without conditioned medium	01-199-1A	500ml
	01-199-1B	100ml

Bone Marrow Karyotyping Medium is intended for use in short-term cultivation of primary bone marrow cells for chromosome evaluation.
Bone Marrow Karyotyping Medium is based on RPMI-1640 basal medium supplemented with L-Glutamine, foetal bovine serum, and antibiotics (penicillin and streptomycin). The medium does not contain any mitogens or conditioned medium.
Bone Marrow Karyotyping Medium is supplied as frozen medium, which is ready for use after thawing.

Instructions for use
The bone marrow karyotyping method was developed to provide information about chromosomal abnormalities. The ready-to-use medium is intended for the culture of bone marrow cells without any mitogens or conditioned medium. After 48-72 hours, a mitotic inhibitor is added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

Storage and Stability
Bone Marrow Karyotyping Medium should be kept frozen at -20ºC. After thawing, the medium should be stored at 2-8ºC. The medium should be used within 10 days after thawing. Protect the medium from light.

Hematopoietic Cell Karyotyping Medium

Product Name	Catalogue No.	Unit Size
Hematopoietic Cell Karyotyping Medium		
With conditioned medium	01-200-1A	500ml
	01-200-1B	100ml

Cytogenetic analysis of human hematopoietic cells using bone marrow aspirates is a standard practice in hematology. Fresh cells or cells grown in short-term cultures often yield an insufficient number of mitotic cells and repeated aspirations are required. Hematopoietic Cell Karyotyping Medium was developed to stimulate the proliferation of human hematopoietic cells from bone marrow as well as peripheral blood.
This medium is particularly effective for karyotyping of acute non-lymphocytic leukemias and various stages of chronic myelogenous leukemia, as well as other hematological disorders such as myelodysplastic syndrome and polycythemia vera. Hematopoietic Cell Karyotyping Medium is based on MEM-Alpha basal medium supplemented with L-Glutamine, foetal bovine serum, antibiotics (penicillin and streptomycin) and conditioned medium.

Hematopoietic Cell Karyotyping Medium is supplied as frozen medium, which is ready for use after thawing.

Instructions for use
The hematopoietic cell karyotyping method was developed to provide information about chromosomal abnormalities. In the presence of a conditioned medium, acute and chronic nonlymphocytic leukemic cells in bone marrow and peripheral blood cultures are stimulated to enter into mitosis by DNA replication. After 48-72 hours, a mitotic inhibitor is added to the culture to stop mitosis in the metaphase stage.
After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

Storage and Stability
Hematopoietic Cell Karyotyping Medium should be kept frozen at -20ºC. After thawing, the medium should be stored at 2-8ºC. The medium should be used within 10 days after thawing. Protect the medium from light.

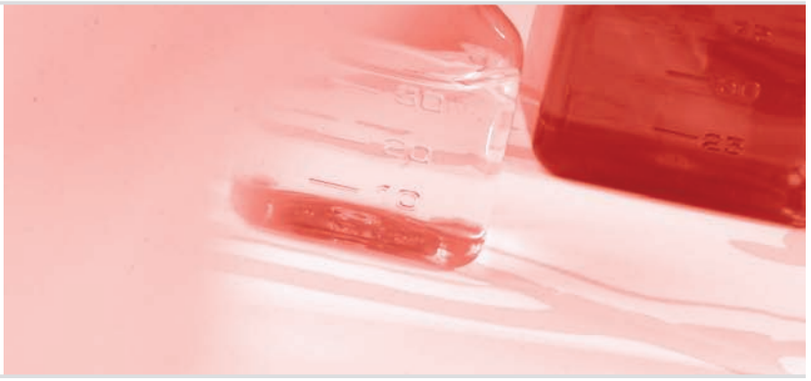
Auxiliary Products

Phytohaemagglutinin M (PHA-M)

Product Name	Catalogue No.	Unit Size
Phytohaemagglutinin M (PHA-M), Lyophilized	12-006-1H	5ml

Phytohaemagglutinin is a lectin extracted from red kidney beans (Phaseolus vulgaris). The protein consists of two molecular species, a leucoagglutinin (PHA-L) and an erythroagglutinin (PHA-E). Each of the proteins contains a family of five isolectins, each being a tetramer held together by noncovalent forces. PHA-M is the mucoprotein form and is a crude extract used for the stimulation of cell proliferation in lymphocyte culture. PHA-M also has a powerful erythroagglutinating property and it was originally used for separating leukocytes from whole blood.
PHA-M from Biological Industries is a sterile, freeze-dried preparation of an aqueous extract from selected red kidney beans. Each bottle should be reconstituted by the addition of 5ml sterile distilled water, using a sterile syringe. After reconstitution, each ml will contain 5-10mg of protein.
Activity: Each lot is tested and standardized for mitotic stimulation using primary human peripheral blood lymphocytes.

Use: 2-4ml of re-hydrated PHA-M per 100ml of culture medium.



Colchicine Solution, 10µg/ml in DPBS

Product Name	Catalogue No.	Unit Size
Colchicine Solution, 10µg/ml in DPBS	12-003-1C	25ml

Colcemid (Demecolcine) Solution, 10µg/ml in DPBS

Product Name	Catalogue No.	Unit Size
Colcemid Solution, 10µg/ml in DPBS	12-004-1D	10ml

Colcemid, N-deacetyl-N-methylcolchicine, is related to colchicine, but animal studies found it to be much less toxic. Colcemid arrests mitotic cultured cells in metaphase and it should be treated with care, since it is mutagenic, tumorigenic, and teratogenic.
Colcemid Solution from Biological Industries is prepared in PBS and it is recommended to use a concentration of 0.1µg/ml in culture medium.
Colcemid is recommended for use in chromosome analysis during lymphocyte karyotyping and amniotic fluid cell chromosome analysis, and in cell synchronization.

Storage
Colcemid Solution should be stored at 2-8ºC, protected from light.

Hypotonic solutions

A major step in harvesting cells for chromosome karyotyping is treatment with a hypotonic saline solution to increase cell volume. Hypotonic solutions work by creating a concentration gradient across the cytoplasmic membrane and water then rushes in by active transport.

Potassium Chloride 0.075 Molar

Product Name	Catalogue No.	Unit Size
Potassium Chloride 0.075 Molar	12-005-1B	100ml

Sodium Citrate Solution (0.8%)

Product Name	Catalogue No.	Unit Size
Sodium Citrate Solution (0.8%)	01-934-1A	500ml

Trypsin EDTA (0.5%), EDTA 0.2% , 10X Conc.

Product Name	Catalogue No.	Unit Size	Formulation Page
Trypsin-EDTA 10X	03-051-5B	100ml	139
	03-051-5C	20ml	139

Giemsa banding has become the most widely used technique for the routine staining of chromosomes. The most commonly used method to obtain this staining is to treat slides with trypsin. This procedure allows for chromosome digestion and high resolution staining.

Trypsin-EDTA 10X from Biological Industries contains Trypsin (1:250) 5gr per liter, and EDTA 2gr per liter, and it should be stored at -20ºC.

Cell Synchronization Kit

Product Name	Catalogue No.	Unit Size
Cell Synchronization Kit	12-008-60	60 reactions

For high-resolution cytogenetic analysis.
The blood cell karyotyping method was developed to provide information about chromosomal abnormalities. Lymphocyte cells do not normally undergo subsequent cell divisions. In the presence of a mitogen, lymphocytes are stimulated to enter into mitosis by DNA replication. After 48-72 hours, a mitotic inhibitor is added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.
High resolution analysis is a special manipulation of the routine blood karyotyping procedure designed to provide a large number of mitotic figures in late prophase or prometaphase. At this stage of mitosis the chromosomes are longer and less condensed. After G-banding, the chromosomes will show greater level of band resolution not seen in routine analysis. High resolution allows more detailed analysis of the karyotype.
Cultures can be synchronized by the addition of methotrexate (MTX), an inhibitor of thymidine biosynthesis which blocks cells in the S-phase (DNA synthesis) of the cell cycle. After 16-18 hours, most of the dividing cells in the cuture are in the S-phase. If thymidine is added to the culture, the MTX block is released and the cells proceed synchronously to mitosis, at which point colcemid may be added. A very short colcemid treatment in conjuction with this technique may be used to produce extended prometaphase chromosomes when small deletions or rearrangements are suspected.

Materials

1. Methotrexate (Amethopterin), 10-5M in HBSS: 4 vials containing 1.5ml each
2. Thymidine, 10-3M in distilled water: 4 vials containing 1.5ml each

Storage and Stability

The solutions must be kept frozen and protected from light. If appropriately stored, the solutions are stable for at least 18 months from the date of preparation.

Product Name	Catalogue No.	Unit Size	Storage Temp.
Prenatal Diagnostics			
BIOAMF-1 Basal Medium	01-190-1A	450ml	2-8ºC
	01-190-1B	90ml	2-8ºC
BIOAMF-1 Supplement	01-192-1D	10ml	-20ºC
	01-192-1E	50ml	-20ºC
BIOAMF-2 Complete Medium	01-194-1A	500ml	-20ºC
	01-194-1B	100ml	-20ºC
BIOAMF-3 Complete Medium	01-196-1A	500ml	-20ºC
	01-196-1B	100ml	-20ºC
Blood Lymphocyte Culture			
Peripheral Blood Karyotyping Medium Without Phytohemagglutinin	01-198-1A	500ml	-20ºC
	01-198-1B	100ml	-20ºC
Peripheral Blood Karyotyping Medium With Phytohemagglutinin	01-201-1A	500ml	-20ºC
	01-201-1B	100ml	-20ºC
	01-201-1H	5ml	-20ºC
Bone Marrow Culture			
Bone Marrow Karyotyping Medium Without conditioned medium	01-199-1A	500ml	-20ºC
	01-199-1B	100ml	-20ºC
Hematopoietic Cell Karyotyping Medium With conditioned medium	01-200-1A	500ml	-20ºC
	01-200-1B	100ml	-20ºC
Auxiliary Products			
Colchicine Solution, 10µg/ml in DPBS	12-003-1C	25ml	2-8ºC
Colcemid Solution, 10µg/ml in DPBS	12-004-1D	10ml	2-8ºC
Potassium Chloride, 0.075 Molar	12-005-1B	100ml	2-8ºC
Phytohemagglutinin-M (PHA-M), Lyophilized	12-006-1H	5ml	2-8ºC
Cell Synchronization Kit	12-008-60	60 reactions	-20ºC
Sodium Citrate Solution (0.8%)	01-934-1A	500ml	AMB
Trypsin EDTA (0.5%), EDTA 0.2% 10X Conc*	03-051-5B	100ml	-20ºC
* see formulation page 137	03-051-5C	20ml	-20ºC

PRODUCTS FOR MALE FERTILITY LABORATORIES

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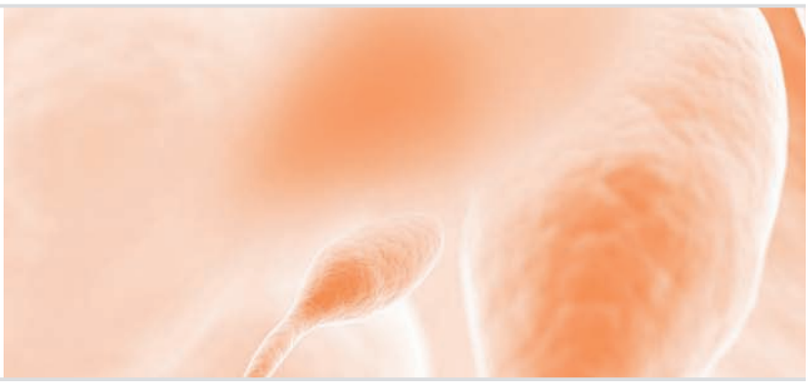
PRODUCTS FOR MALE FERTILITY LABORATORIES



Modified Ham's F-10 with Gentamicin

Product Name	Catalogue No.	Unit Size
Modified Ham's F-10 With Gentamicin	01-925-1B	100ml

Designed for in-vitro sperm washing procedures which do not require CO_2 incubation. Modified Ham's F-10 contains HEPES and Sodium Bicarbonate. This buffering system provides optimum pH maintenance (7.2-7.4) and does not require the use of a CO_2 incubator.

Contains
Ham's F-10 supplemented with:
- 21mM HEPES
- 4mM Sodium Bicarbonate
- Human Serum Albumin (for human use, USP)
- Sodium Lactate
- Gentamicin
- Phenol Red

Protein supplementation
The medium contains 0.6% Human Serum Albumin (HSA). The HSA used in the manufacture of this medium is approved for human use.
See Chapter 19 - Human Blood for HSA Product.

Storage
Store at 2-8ºC.

Quick Stain

Product Name	Catalogue No.	Unit Size
Quick Stain	01-939-1U	3ml for 200-300 slides

A dye mixture for staining and visualization of semen specimens.

Quick Stain is an in-vitro diagnostic rapid test for spermatozoa staining and morphology assessment. It also permits classification of round cells in semen -mainly immature germ cells and leukocytes. Excessive numbers of leukocytes may be associated with infection and poor sperm quality.
With Quick Stain, nuclei are stained dark-purple while acrosome, tail and other cell structures have different shades of violet.

Storage
The stain solution bottle should be kept tightly closed.
Quick Stain should be protected from light and stored at 2-8ºC.


BIOLOGICAL INDUSTRIES
KIBBUTZ BEIT HAEMEK ISRAEL
STERILE
CE
MODIFIED F-10 (HAM)
with antibiotics
Store:+2 to 8°C
Cat. 01-925-1B
Vol. 100 ml
Exp: 09/2010
Lot: 912436
For In Vitro Diagnostic Use
www.bioind .com

MYCOPLASMA– DETECTION & TREATMENT

13

MYCOPLASMA-
DETECTION & TREATMENT

Mycoplasma Detection

Mycoplasma is a prokaryotic microorganism of the class Mollicutes that lack a true cell wall, and many of which are considered pathogenic.
Mycoplasma contamination is often detected in cell cultures, and consequently, virus cultures, vaccines and other biological materials produced in cells become contaminated as well.
Mycoplasma contamination in cell lines used for research poses a serious problem. In most cases, visual detection of such contaminations or detection with the aid of a microscope is impossible. Although mycoplasma does not cause visible damage to cells, it undeniably affects cell metabolism, cell growth in culture, protein synthesis, cytokine secretion, and even causes damage to DNA and RNA. Hence, results obtained from experiments are liable to be biased when mycoplasma is present. Various studies show that the percentage of contaminated cultures in cell banks is 10%-80%. Mycoplasma contamination can originate from bovine serum, laboratory employees, other contaminated cultures, or the animals from which the cells have been harvested.
The most prevalent species of mycoplasma detected in contaminated cell cultures include M. fermentans, M. hyorhinis, M. arginini, M. orale, M. salivarium, M. hominis, M. pulmonis and M. pirum.

Testing Methods
Several methods for the detection of mycoplasma have been published:

- Cultures on agar, liquid media, or semi-solid media.
- DAPI Staining – staining DNA with fluorescent dyes (4', 6-diamine-2 phenylindole dihydrochloride).
- DNA hybridization.
- Antibodies for specific mycoplasma species.
- Electronic microscope.
- PCR: specific primers.
- Biochemical – detection of mycoplasmal enzymes by colorimetric or luminescence assay.

Using PCR for the Detection

The testing required by the regulatory authorities is seeding in culture (agar and liquid media). This test is complicated, time consuming (about 5 weeks), and some mycoplasma species are difficult to detect with this method. In recent years, the disadvantages of these methods have been acknowledged (such as sensitivity, specificity and long and complex procedures), and use of PCR for the detection of contaminations in cell cultures has become increasingly widespread.
PCR has been shown to be a highly sensitive, specific and rapid method for the detection of mycoplasma contamination in cell cultures. Specific primers have been designed from DNA that is coded to the ribosomal RNA (16SrRNA). The gene sequences for RNA are considered conserved sequences and are similar in the various mycoplasma species, which have not undergone significant mutation. Consequently, primers can be designed for these areas, which are specific to the mycoplasma and will not detect bacterial or animal DNA sequences.
The literature describes several PCR methods for the detection of mycoplasma, such as using a number of primers to obtain detection of specific mycoplasma species, and nested PCR (two consecutive PCR cycles using different primers) for amplifying sensitivity and specificity.
PCR testing techniques are based on amplification of a DNA fragment using primers prepared in advance, and fragment identification is usually carried out with electrophoresis.
In conjunction with Prof. Shlomo Rottem of the Mycoplasma Laboratory at the Hebrew University-Hadassah Medical School, Jerusalem, Biological Industries has developed the EZ-PCR Mycoplasma Test Kit (Cat. # 20-700-20; 20-700-10) a PCR-based mycoplasma test kit that simplifies testing and detection of mycoplasma contamination in cell cultures. The kit includes a unique reaction mix that contains all the ingredients required for PCR: nucleotides, primers, Taq Polymerase and magnesium. No prior preparations are required for PCR, other than the sample to be tested (centrifugation and suspension in the buffer supplied with the kit). After performing agarose gel electrophoresis, positive samples will yield a 270bp fragment. The test takes approximately five hours to complete.

The primers have been designed to detect the mycoplasma species responsible for most contaminations in cell cultures (including Acholeplasma). The primers were tested and found to be specific to mycoplasmatic DNA, and do not react with animal or bacterial DNA.

In sensitivity tests for the detection of defined mycoplasmas, the EZ-PCR Mycoplasma Test Kit was found to be very sensitive in comparison to other test kits currently available on the market (Table 1). The ability to routinely conduct rapid and simple tests to detect mycoplasma contamination in cell cultures facilitates the eradication or treatment of contaminated cells.

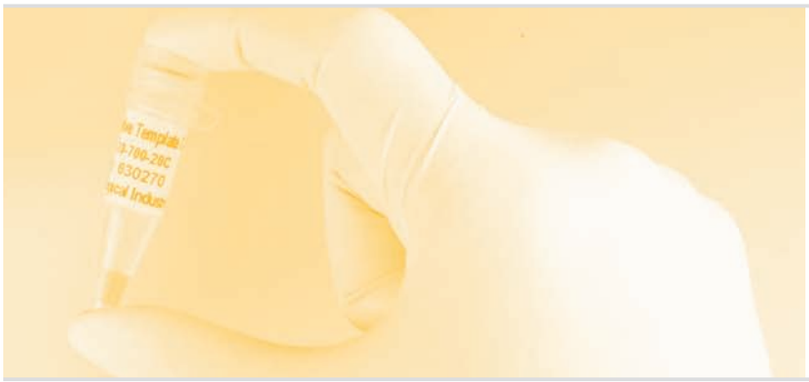


Table 1: Minimal concentration of mycoplasma detected with EZ-PCR Mycoplasma Test Kit

	Without Sample Preparation	After Sample Preparation (conc. 1/20)*
M. fermentans	240 CFU/ml	12.00 CFU/ml
M. capricolum	110 CFU/ml	5.50 CFU/ml
M. penetrans	200 CFU/ml	16.66 CFU/ml
M. hyorhinis	210 CFU/ml	10.50 CFU/ml

* According to EZ-PCR Mycoplasma Test Kit instructions.

EZ-PCR Mycoplasma Test Kit

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ-PCR Mycoplasma Test Kit	20-700-10	10 assays	-20°C
	20-700-20	20 assays	-20°C

Ready-to-use PCR Mix for the detection of mycoplasma in cell culture

EZ-PCR Mycoplasma Test Kit is designed to detect the presence of mycoplasma in contaminated biological materials, such as cultured cells. Mycoplasma detection by the direct culture procedure is time-consuming and some mycoplasma species are difficult to cultivate. With PCR testing, results are obtained within a few hours, since the presence of contaminant mycoplasma can be easily detected simply by verifying the bands of amplified DNA fragments in electrophoresis. There is no need to prepare probes labeled with radioisotopes, or to calculate enzyme, dNTPs or buffer concentrations. Instead, a ready-to-use, optimized PCR mix is supplied. The reaction mix contains a precipitant for direct loading of PCR products onto agarose gel. The primer set allows detection of various mycoplasma species (M. fermentans, M. hyorhinis, M. arginini, M. orale,M. salivarium, M. hominis, M. pulmonis, M. arthritidis, M. bovis, M. pneumoniae, M. pirum and M. capricolum), as well as Acholeplasma and Spiroplasma species, with high sensitivity and specificity.

Advantages

EZ-PCR Mycoplasma Test kit is based on a simple assay protocol and has the following advantages:

- Highly sensitive.
- Mycoplasma-specefic primers with broad range.
- Convenient and user-friendly: supplied with complete reaction mix (with Taq polymerase). Requires no more than 10-20 minutes of actual work.
- Samples are easy to prepare.
- Results are easily determined with a single PCR process.
- Rapid: results obtained in no more than 5 hours.
- No loading dye needed for the agarose gel.

Kit Components

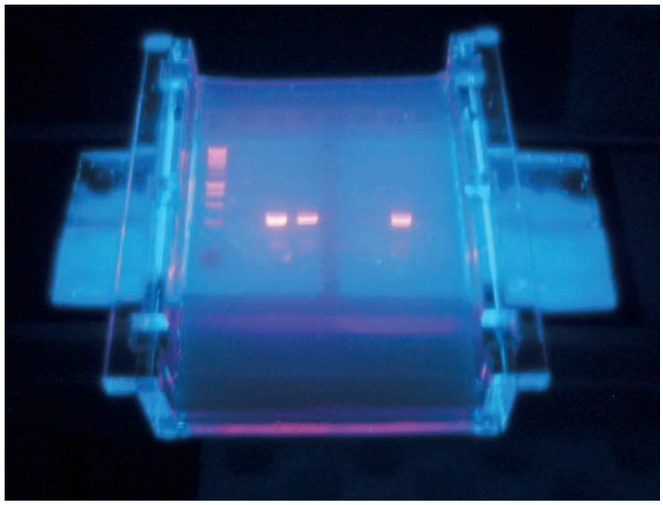
Kit Components	EZ-PCR Mycoplasma Test kit Catalogue No. 20-700-10	EZ-PCR Mycoplasma Test kit Catalogue No. 20-700-20
Reaction Mix	100µl	200 µl
Buffer Solution	0.5ml	1ml
Positive Template Control	10µl	20µl

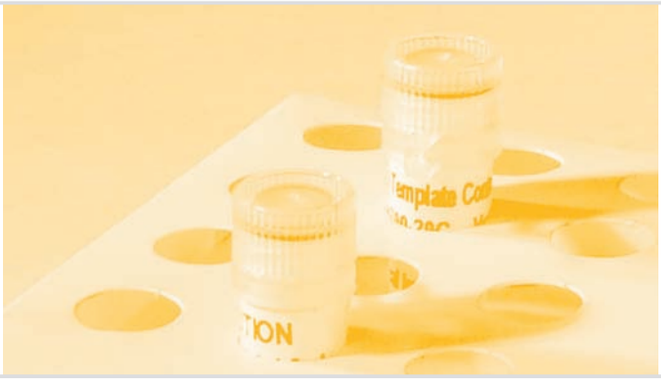
Principle

rRNA gene sequences of prokaryotes, including mycoplasmas, are well conserved, whereas, the lengths and sequences of the spacer region in the rRNA operon (for example the region between 16S and 23S gene) differ from species to species.

The detection procedure utilizing the PCR process with this primer set consists of:

1. Amplification of a conserved and mycoplasma-specific 16S rRNA gene region using two primers.
2. Detection of the amplified fragment by agarose gel electrophoresis. This system does not allow the amplification of DNA originating from other sources, such as tissue samples or bacteria, which affect the detection result. Amplification of the gene sequence with PCR using this primer set enhances not only the sensitivity, but also the specificity of detection. Amplified products are then detected by agarose gel electrophoresis.





Treatment of Mycoplasma-Infected Cells with Antibiotics

Using Antibiotics to Disinfect Cell Cultures

The increasingly widespread use of more sophisticated and sensitive methods for the detection of mycoplasma contamination in cell cultures has resulted in contamination being detected in numerous cultures. This raises the issue of how to eliminate mycoplasma contamination. Naturally, the ideal solution is to discard the contaminated cells. However, if the cells that are stored in liquid nitrogen are also contaminated, a solution is required for eliminating the mycoplasma and preparing a new cell bank, particularly if the cells are unique and the result of extensive work.

A number of effective methods for the elimination of mycoplasma contamination in cell cultures have been published, such as:

- Treatment with specific hyperimmune serum (antibodies).
- Passage of contaminated cells in thymus-deficient mice.
- Exposure to analogs of nucleic acids that prevent reproduction of mycoplasma.
- Treatment with antibiotics.
- Exposure of contaminated cells to mouse macrophages.
- A technique that combines growing cells on soft agar and treatment with antibiotics.

The preferred method in terms of simplicity is treatment with antibiotics, which do not damage or alter cells. Antibiotics such as penicillin, which attacks bacterial cell walls, are ineffective in this instance, since mycoplasma lacks a true cell wall. Several antibiotics eliminate mycoplasma effectively, such as Tylosin, Neomycin, Tetracycline and Gentamycin. However, the efficacy of these antibiotics is restricted to specific mycoplasma species and frequently only reduces the concentration of mycoplasmas, rather than disinfect the cell culture. Consequently, as soon as treatment is concluded, contamination will recur.
Two methods are recommended for treating contaminated cells with antibiotics. The first is based on alternating treatment with two types of antibiotics (Tiamutin and Minocycline), and the second on treatment with one type of antibiotic (Ciprofloxacine).

Summary

Heightened awareness regarding mycoplasma contamination, and increased use of sensitive and effective methods for the detection and treatment of mycoplasma contaminations, will lead to a reduction in the percentage of contaminated cultures. In addition to isolating contaminated cultures, and discarding or treating them, meticulous work procedures should be followed, and only mycoplasma-free raw materials should be used.

The contamination of cells with mycoplasma is a very common problem, although it often goes unnoticed since no cloudiness appears in the cell culture. Nevertheless, the contamination often causes biochemical changes as well as changes in the immunological properties of the cells.
Since mycoplasma-infected cells cannot always be discarded, many complex methods have been suggested for the elimination of mycoplasma.

Biological Industries is now offering a combination of antibiotics, which have been shown to be effective in the elimination of mycoplasma species that account for 90% of the contamination found in cell cultures.
When used according to the following instructions, no cytotoxic effects will occur.

BIOMYC-1 & BIOMYC-2

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOMYC-1 Antibiotic Solution 100X Conc.	03-036-1B	100ml	-20°C
	03-036-1C	20ml	-20°C
	03-036-1D	10ml	-20°C
BIOMYC-2 Antibiotic Solution 100X Conc.	03-037-1B	100ml	-20°C
	03-037-1C	20ml	-20°C
	03-037-1D	10ml	-20°C

BIOMYC-1 is based on the antibiotic Tiamutin, which is produced by the fungus pleurotus mutilus. BIOMYC-2 is based on minocycline, which is a tetracycline derivative. These two antibiotic solutions are generally used sequentially in combination.

Instructions for Use

1. Do not use the two solutions together, but sequentially.
2. Add 1ml BIOMYC-1 to 100ml medium, and maintain the contaminated cells in this mixture for 4 days. Any fresh medium added should also contain BIOMYC-1.
3. After 4 days, add 1ml BIOMYC-2 to 100ml fresh medium, and maintain the cells in this second mixture for 3 days.
4. The above, together, are considered as one treatment cycle. It may be necessary to repeat this cycle 2-3 times.
5. During the process, the cells can be tested for mycoplasma contamination, and results can then be used to shorten the process when possible.

BIOMYC-3

Product Name	Catalogue No.	Unit Size	Storage Temp.
BIOMYC-3 Antibiotic Solution 100X Conc.	03-038-1B	100ml	-20°C
	03-038-1C	20ml	-20°C
	03-038-1D	10ml	-20°C

BIOMYC-3 is based on the ciprofloxacin antibiotic, which is a member of the fluoroquinolone group. Many mycoplasma species have been found to be sensitive to BIOMYC-3, including A.laidlawii, M.orale, M.hyorhinis, M.fermentans and M.arginini. These species are responsible for most of the contamination in cell culture[1]. At the recommended concentrations, no cytotoxic effects have been found, and the treatment is quite easy to perform.

Instructions for Use

1. Add 1ml BIOMYC-3 to 100ml medium.
2. Continue the treatment for a total of 14 days, changing the medium (containing BIOMYC-3) every 2-3 days.
3. Retain the cells in the growth medium for an additional 14 days before re-testing for mycoplasma.

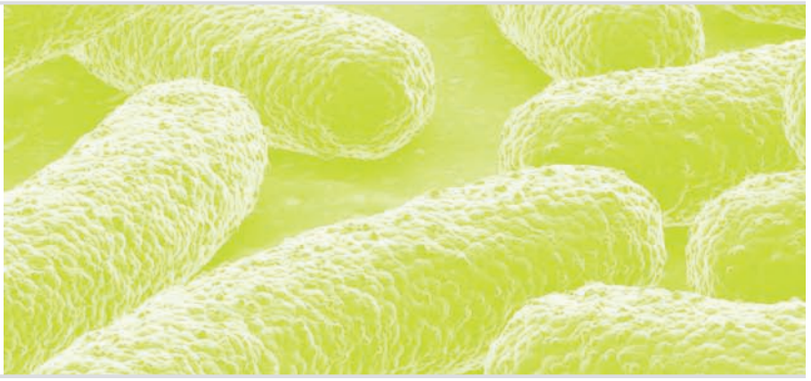
(1) Schmitt, k. et al., J. Immunol. Methods, 109: 17-25 (1988)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Mycoplasma Detection			
EZ-PCR Mycoplasma Test Kit	20-700-10	10 assays	-20°C
	20-700-20	20 assays	-20°C
Treatment of Mycoplasma-Infected Cells with Antibiotics			
BIOMYC-1 Antibiotic Solution 100X Conc.	03-036-1B	100ml	-20°C
	03-036-1C	20ml	-20°C
	03-036-1D	10ml	-20°C
BIOMYC-2 Antibiotic Solution 100X Conc.	03-037-1B	100ml	-20°C
	03-037-1C	20ml	-20°C
	03-037-1D	10ml	-20°C
BIOMYC-3 Antibiotic Solution 100X Conc.	03-038-1B	100ml	-20°C
	03-038-1C	20ml	-20°C
	03-038-1D	10ml	-20°C

DISINFECTANTS

14

DISINFECTANTS



The term 'disinfection' as denoted here refers to the destruction of potentially pathogenic microorganisms on inanimate objects. Disinfectants are basically chemical agents that are either lethal to microorganisms or inhibit their microbial activity and growth. Chemical disinfection plays a very crucial role, especially in the laboratory setting. Microorganisms, the cause of infectious diseases, are a heterogeneous group of organisms with a wide range of characteristics. With such a wide spectrum of biological characteristics, it is to be anticipated that particular groups of microorganisms or strains within a group will respond differently to the various means of disinfection. Disinfection is only one of the variegated measures that can be utilized to prevent and/or break the cycle of disease, but if used alone it is often adequate. Good hygiene and sanitation practices within the work setting are essential if the disinfection process is to be effective.
Some of the modes of action of disinfectants include:

- Adversely affecting the microbe's cellular physical structure by disruption of intermolecular interactions
- Causing ensuing dissociation of the cellular bilayers
- Compromising cellular permeability and/or
- Inducing leakage of cellular contents by enzymatic dissociation
- Deactivation of other cellular metabolic processes.

An understanding of the basic principles involving the disinfection process is essential to achieve a satisfactory outcome. The selection and use of any chemical disinfectant requires a detailed knowledge of such factors, such as:

- Safety (i.e., potential hazards and toxicity)
- Range/spectrum of activity
- Ambient room temperature and humidity
- Type of surface
- Effectiveness in the presence of organic matter, lipids, fatty-acids and proteins
- Stability and reactivity
- Neutralization by pH changes
- Neutralization by soaps or detergents
- Water hardness
- Contact time
- Specificity
- Environmental considerations

The aim of chemical disinfection is not to sterilize surfaces but rather to reduce the extent of microbial contamination to the lowest possible level. Most people utilizing disinfectants appreciate how essential it is to practice pristine hygiene and sanitation protocols, especially in a laboratory setting. Remember that disinfectants do not act instantaneously. Destruction of pathogens occurs in three phases:

1. Initial/Lag Phase - When the disinfectant starts showing activity
2. Median Phase - When the majority, but not all the microorganisms might be killed
3. Final Phase - When the more resistant microorganisms are destroyed.

As a rule, allow the disinfection process the necessary time to optimize its biocidal activity.

Biological Industries offers a variety of effective disinfectant agents. The best choice of product depends on the needs of the particular application and includes the following products:

Pharmacidal

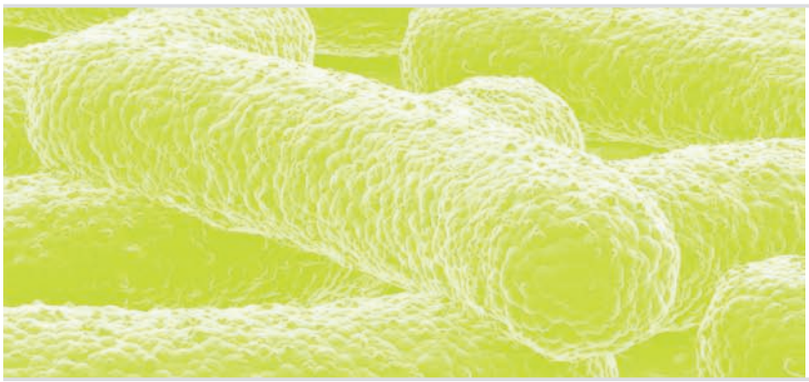
Product Name	Catalogue No.	Unit Size	Storage Temp.
Pharmacidal, spray bottle for disinfecting surfaces	IC-110100	1 liter	AMB
	IC-110100-B	100ml	AMB
Pharmacidal, for disinfecting surfaces (without sprayer)	IC-110100-G	5 liter	AMB
	IC-110100-L	250ml	AMB

Disinfectant solution for incubators and sterile benches in cell culture and molecular biology laboratories.

The problem of contamination in incubators and/or sterile workbenches is often serious, leading to extensive damage. Pharmacidal solution prevents contamination, growth of fungi (and spores), bacteria (and spores) mycoplasma and viruses (including HIV and Hepatitis B).
The active ingredients are quaternary benzylammonium compounds, and the solution does not contain mercury, formaldehyde, phenol or alcohol. Furthermore, Pharmacidal is non-toxic and biodegradable. It has also been found to be fully compatible with common work surfaces.

Recommended use

- Spray incubators once every 2 weeks.
- Spray sterile benches once a day, or preferably before each laboratory worker begins using the work area.
- The surfaces to be disinfected should be completely wet by spraying. Allow to dry, no rinsing necessary.



Aquaguard-1

Product Name	Catalogue No.	Unit Size	Storage Temp.
AQUAGUARD-1 Solution for disinfecting water baths of CO_2 incubators	01-867-1B	100ml	-20°C

100ml of 100X concentrated solution for disinfecting CO_2 water baths. (Use 50ml per 5 liters of water in bath).

The water required for humidity is a source of contamination that disperses in the incubator. In order to disinfect the water we recommend Aquaguard-1 Solution, which contains a disinfectant that does not cause damage to the stainless steel tray, is non-toxic, non-volatile, and extremely effective. The water should be replaced with sterile water every two to four weeks, adding 50ml of Aquaguard-1 per 5 liters of water.

Preventive treatment as described above will prevent damage that can be caused as a result of contamination to the tissue culture. In addition, it will also prevent the necessity of dealing with contamination that has dispersed in the incubator and causes repeated contamination of the tissue culture.

Aquaguard-2

Product Name	Catalogue No.	Unit Size	Storage Temp.
AQUAGUARD-2 Solution for disinfecting ordinary water baths	01-916-1E	50ml	-20°C

50ml of 500X concentrated solution for prevention of microbial growth in water baths. (Use 2ml per 1 liter of water).

Aquaguard-2 is intended for disinfecting various kinds of water baths from bacteria and fungi. It is recommended to use 2ml of Aquaguard-2 for each liter of water in the bath, and to repeat the procedure every 1-2 weeks. After 4-6 weeks, the bath should be emptied and refilled with water containing Aquaguard-2.

CELL VIABILITY

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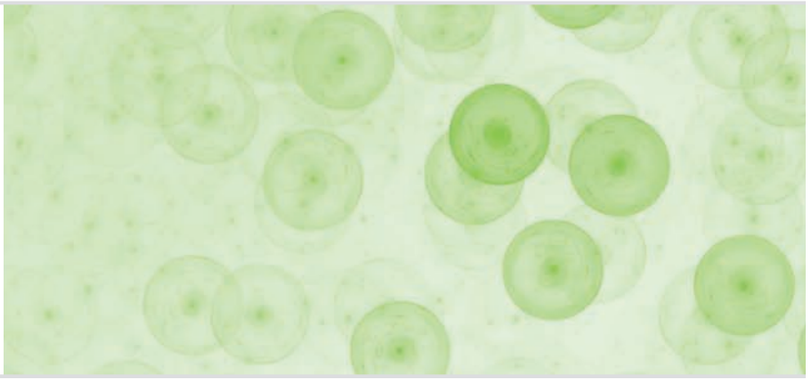
CELL VIABILITY



Cell Proliferation Kit (XTT Based)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Cell Proliferation Kit (XTT based)	20-300-1000	1000 assays	-20°C

Cell proliferation assays are widely used in cell biology for the study of growth factors, cytokines and media components, for the screening of cytotoxic agents and for lymphocyte activation.

The need for a reliable, sensitive and quantitative assay that would enable analysis of a large number of samples led to the development of methods, such as:

- Use of radioactive thymidine to label DNA in live cells
- Use of Brdu to label DNA in live cells (as a substitute for radioactive thymidine)

The above methods have a number of disadvantages, including: use of radioactive materials and relatively complex techniques. The use of tetrazolium salts, such as MTT, is based on the fact that live cells reduce tetrazolium salts into colored formazan compounds. The biochemical procedure is based on the activity of mitochondria enzymes which are inactivated shortly after cell death. This method was found to be very efficient in assessing the viability of cells.

A colorimetric method based on the tetrazolium salt, XTT, was first described by P.A. Scudiero in 1988. Whilst the use of MTT produced a non-soluble formazan compound which necessitated dissolving the dye in order to measure it, the use of XTT produces a soluble dye.
The use of XTT greatly simplifies the procedure of measuring proliferation, and is, therefore, an excellent solution to the quantitating of cells and their viability without using radioactive isotopes. This kit was developed to assay cell proliferation in reaction to different growth factors, cytokines and nutrient components. In addition, it is suitable for assaying cytotoxicity of materials such as TNF or other growth inhibitors. XTT can be used as a non-radioactive substitute for cytotoxic tests based on the release of 51Cr from cells with no less sensitivity.

Advantages:

- Easy-to-use: there is no requirement for additional reagents and/or the cell washing procedures
- Speed: multiwell plates and an ELISA reader can be used for reading
- Sensitivity: can be assayed even in low cell concentrations
- Accuracy: dye absorbance is proportional to the number of cells in each well
- Safety: there is no need for radioactive isotopes

Kit Components

- XTT Reagent (10x5ml), a sterile solution containing the XTT reagent. The solution should be stored frozen and should not be exposed to light. To avoid repeated re-freezing, dividing the solution into a number of vials after defrosting the original vial is recommended.
  Note: if sediment is present in the solution, heat the solution to 37°C and swirl gently until a clear solution is obtained.
- Activation Reagent (2x0.5ml), a sterile solution containing PMS (N-methyl dibenzopyrazine methyl sulfate). The solution should be stored frozen and should not be exposed to light. To avoid repeated re-freezing, dividing the solution into a number of vials after defrosting the original vial is recommended.
  Note: if sediment is present in the solution, heat the solution to 37°C and swirl gently until a clear solution is obtained.

Assay Principles

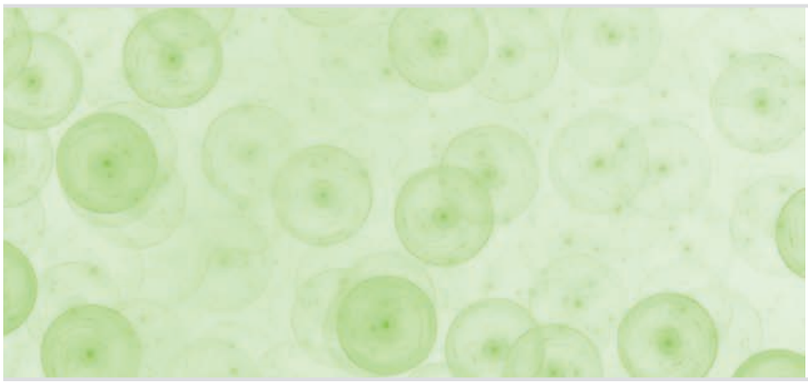
The assay is based on the ability of metabolic active cells to reduce the tetrazolium salt XTT to orange colored compounds of formazan. The dye formed is water soluble and the dye intensity can be read at a given wavelength with a spectrophotometer. The intensity of the dye is proportional to the number of metabolic active cells. The use of multiwell plates and an ELISA reader enables testing a large number of samples and obtaining easy and rapid results. The test procedure includes cultivation of cells in a 96-well plate, adding the XTT reagent and incubation for 2-24 hours. During incubation an orange color is formed, the intensity of which can be measured with a spectrophotometer, in this instance with an ELISA reader. The greater the number of active cells in the well, the greater the activity of mitochondria enzymes, and the higher the concentration of the dye formed, which can then be measured and quantitated.

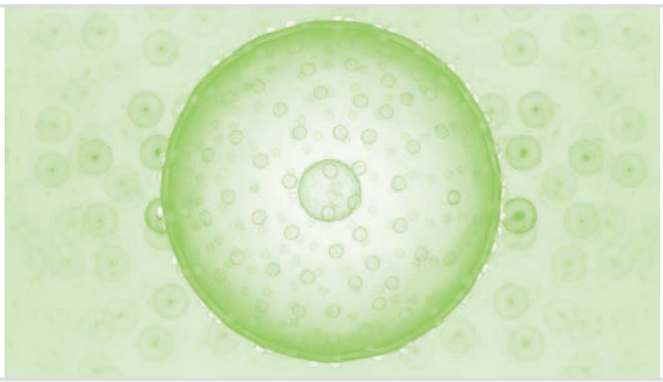
Typical Experiment: The Cytotoxicity of Butylated Hydroxyanisole (BHA)

Butylated Hydroxyanisole (BHA)- synthetic antioxidant used in the food and cosmetic industry.

Mechanism of cytotoxicity

Low doses of BHA exerted a significant cytotoxic effect, associated with loss of mitochondrial function. As the concentration of BHA increases, morphological alterations in critical sub-cellular targets such as lysosomes, mitochondria and actin cytoskeleton, are observed. In parallel, BHA induced an irreversible loss of cell proliferative capacity, preceding apoptosis induction.





The cytotoxic system
Vero cells were exposed to increased concentrations of BHA (0-500μM) for 24 hours to create a cytotoxic system.

BHA cytotoxicity of Vero cells
Vero cells were cultured (5000 cells per well) in a 96 well plates for 24 hours. Cells were exposed to increased concentrations of BHA (0-500μM) for 24 hours, then viability was measured, using a colorimetric method (XTT Based Cell Proliferation Kit , Cat. No. 20-300-1000). XTT reagent was added and absorbance was measured (wavelength of 450nm and reference of 690nm) after a further 5 hours of incubation.

Figure 1: Determination of the cytotoxicity activity of BHA on vero cells.

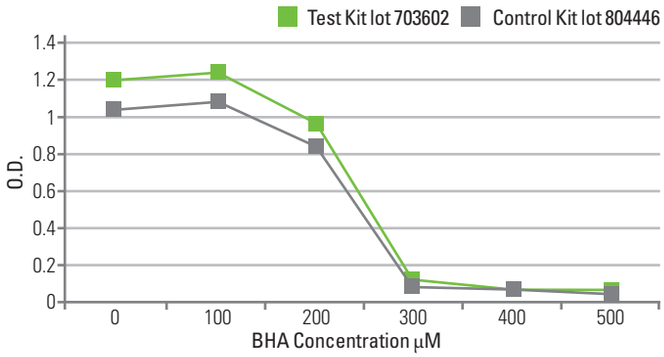
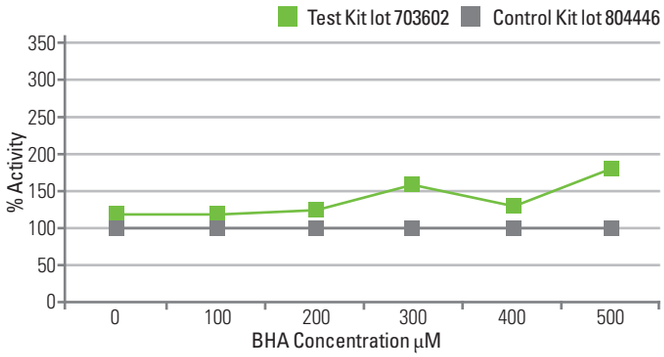


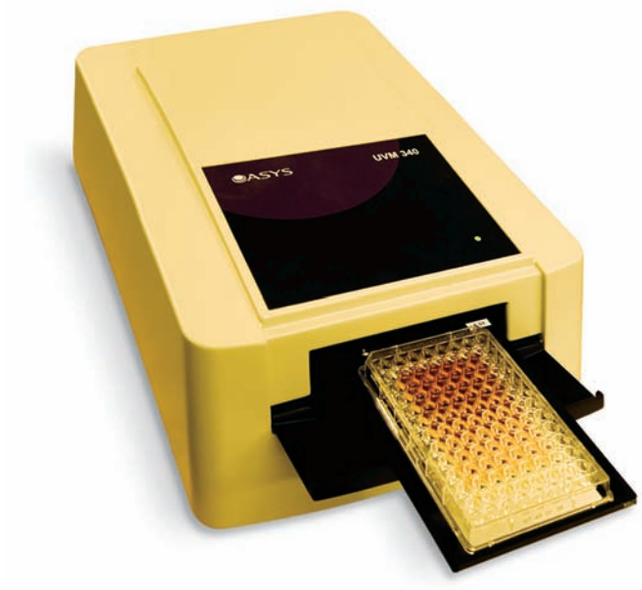
Figure 2: % Activity using test and control kit.
Each point of the control kit was defined as 100% activity. The % activity of the test kit is presented as percentage from the control kit at the same point.



Trypan Blue (0.5% Solution)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Trypan Blue Solution 5mg/ml in Saline	03-102-1B	100ml	AMB

Trypan Blue is the stain most commonly used to distinguish viable from nonviable cells. Viable cells exclude the dye, while nonviable cells absorb the dye and appear blue. Cells should be in suspension as single cells in buffered saline before counting.
Trypan Blue has a higher affinity for serum protein than for cellular proteins, so suspending cells in medium containing serum will generate a dark background.



MOLECULAR BIOLOGY

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MOLECULAR BIOLOGY

EZ-RNA Total RNA Isolation Kit

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ-RNA Total RNA Isolation Kit	20-400-100	100ml	2-8ºC

EZ-RNA is a complete kit with ready-to-use reagents for the isolation of total RNA from samples of human, animal, plant, yeast, bacterial and viral origin. EZ-RNA is based on disruption of cells in guanidinium thiocyanate/ detergent solution, followed by organic extraction and alcohol precipitation of the RNA, and which allows simultaneous processing of a large number of samples. The resulting RNA is suitable for the isolation of Poly A+ RNA or for Northern Blotting, Dot Blotting, or other analytical procedures. DNA and proteins can also be recovered from the interphase and the organic phase of the same sample.

Kit reagents

- Denaturing Solution, 50ml, contains: Guanidine Thiocyanate
- Extraction and Phase Separation Solution, 50ml, contains: phenol and chloroform

EZ-RNA II Total RNA Isolation Kit

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ-RNA II Total RNA Isolation Kit Without Chloroform With BCP	20-410-100	100ml	2-8ºC

With BCP instead of chloroform

EZ-RNA is a complete kit with ready-to-use reagents for the isolation of total RNA from samples of human, animal, plant, yeast, bacterial and viral origin. EZ-RNA II is based on disruption of cells in guanidine thiocyanate/ detergent solution, followed by organic extraction and alcohol precipitation of the RNA, and which allows simultaneous processing of a large number of samples. 1-Bromo-3-chloropropane (BCP) replaces chloroform, a highly volatile and toxic reagent used in molecular biology. Substituting BCP for chloroform in the EZ-RNA II kit reduces toxic material handling without any adverse effects on the quality of isolated RNA, DNA or proteins. The resulting RNA is suitable for the isolation of Poly A+ RNA or for Northern blotting, dot blotting, in vitro translation, molecular cloning, RT-PCR and RNase protection assays, or other analytical procedures. DNA and proteins can also be recovered from the interphase and the organic phase of the same sample.

Kit reagents

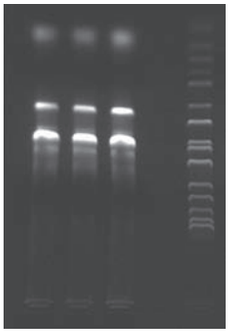
- Denaturing Solution, 50ml, contains: Guanidine Thiocyanate
- Water-saturated phenol, 40ml
- 1-Bromo-3-chloropropane (BCP), 9ml

Assessing yield of total RNA

The yield of total RNA will vary depending on the tissue or cells from which it is obtained.

Tissue/sample type	Amount of starting material	Yield of total RNA
Rat liver	1mg	6µg
Rat skeletal muscle	1mg	0.9µg
Mouse brain	1mg	1.25µg
Mouse spleen	1mg	2.5µg
Mouse testes	1mg	2.5µg
Mouse thymus	1mg	0.85µg
Human cerebellum	1mg	0.8µg
Human prostate tumor	1mg	1µg
MCF-7 cell line	10^8 cells	720µg
U251 cell line	10^8 cells	950µg
Kidney	1mg	3µg
Placenta	1mg	1-4µg
Epithelial cells	10^6 cells	8-15µg
Fibroblast cells	10^6 cells	5-7µg
Plant poinsettia	1mg	0.7µg
Tobacco	1mg	0.8µg
Yeast	10^7 cells	1-5µg
Bacteria	10^9 cells	3-5µg

Figure 1: Total RNA extracted from hybridoma cells



RNA Save

Product Name	Catalogue No.	Unit Size
RNA Save	01-891-1A	500ml
	01-891-1B	100ml
	01-891-1C	20ml

Tissue storage solution for RNA stabilization

RNA Save is an aqueous, non toxic, tissue and cells storage solution intended for the preservation of RNA for later isolation. Samples in RNA Save solution can be stored indefinitely at -20°C or -80°C with no RNA degradation. RNA save solution can be used for the storage of tissues, cells, bacteria and yeasts. The solution may not be effective for the storage of waxy plant tissue and bone because of poor penetration of the solution. RNA Save is compatible with most RNA isolation methods.

Storage
RNA Save should be stored at room temperature. If precipitation is seen, warm the solution to 37°C and mix carefully for resolubilization.

RNase-ExitusPlus™

Product Name	Catalogue No.	Unit Size
RNase-ExitusPlus™	01-897-1L	250ml
	01-897-1B	100ml

A solution for the decontamination of RNase

RNase-ExitusPlus™ is a non-alkaline, non-corrosive and non-carcinogenic cleansing solution that is highly active against RNase contamination. RNase-ExitusPlus™ has been demonstrated to inactivate more than 20µg of RNase A dried onto the bottom of a microcentrifuge tube. RNase-ExitusPlus™ is heat resistant.

Features
These are the new and unique characteristics of RNase-ExitusPlus™:
- Catalytic and cooperative effects of the components cause a very rapid nonenzymatic, non-sequence-specific degradation of protein and RNase molecules.
- All components of RNase-ExitusPlus™ are readily biologically degradable and not harmful or toxic for humans.
- No aggressive mineral acids or alkaline substances are used. Equipment and materials are not damaged or corroded even after prolonged incubation time.
- No organic solvents or volatile components, no toxic fumes.
- Elevated temperatures above approx. 50°C speed up the reaction and the efficiency/activity!

RNase-ExitusPlus™ is ready-to-use for eliminating RNase from any surface including the interior of microcentrifuge tubes. By following a few simple decontamination instructions, RNase is completely inactivated and removed.

Storage
RNase-ExitusPlus™ should be stored at room temperature; at colder temperatures a precipitate may form which is easily brought into solution at 37°C.

DNA-ExitusPlus™

Product Name	Catalogue No.	Unit Size
DNA-ExitusPlus™	01-898-1L	250ml
	01-898-1B	100ml

Solution for the removal of DNA and RNA contaminations

DNA amplification is one of the most commonly used techniques in the modern research laboratory. The presence of contaminating DNA in and around PCR workstations can lead to unwanted artifacts during amplification.

Principally, there are two ways to make DNA not amplifiable:
1. By degradation of the DNA (e. g. by the addition of DNases or chemical destruction), or
2. By modification of bases - leaving the DNA strand intact, but blocked for reading by polymerases.

Using a DNA strand break assay it has been shown that not all DNA decontamination solutions on the market totally degrade DNA! DNA-ExitusPlus™ is an improvement over those products and causes both strand breakages as well as degradation. When used properly at the work area, it totally eliminates the amplification of non-target DNA. DNA-ExitusPlus™ is a non-alkaline, non-corrosive and non-carcinogenic cleansing solution which is effective on all surfaces. A severe disadvantage of conventional decontamination reagents is revealed in a new test for the corrosive potential of their components. For this purpose, different metal plates were incubated

for 20 minutes with identical aliquots of the reagents. The selected metals are representative for equipment and materials found in laboratories.
The results of this corrosion test were that all known commercial products contain aggressive chemical substances with corrosive, harmful or even toxic effects. These conventional reagents are known to contain azides, mineral acids like phosphoric acid or hydrochloric acid, aggressive peroxides or strong alkaline substances like sodium hydroxide. Even after an incubation of only 20 minutes, irreversible damages of the metal surfaces are observed in many cases. The newly developed solution DNA-ExitusPlus™ exhibits its unique characteristics especially in this corrosion test. For all metal surfaces tested, no damage or corrosion is observed after treatment. DNA-ExitusPlus™ was also tested under identical conditions on many different plastic surfaces without any indications of damage.
The reagent is heat stable.

Features
These are the new and unique characteristics of DNA-ExitusPlus™:
- Catalytic and cooperative effects of the components cause a very rapid nonenzymatic, non-sequence-specific degradation of DNA and RNA molecules.
- All components of DNA-ExitusPlus™ are readily bio-degradable and not harmful or toxic for humans.
- No aggressive mineral acids or alkaline substances are used. Equipment and materials are not damaged or corroded even after prolonged incubation times.
- No organic solvents or volatile components, no toxic fumes.
- Elevated temperatures above approx. 50°C speed up the reaction and the activity.

Storage
DNA-ExitusPlus™ should be stored at room temperature.
The reagent contains component that will precipitate below temperatures of 15°C. Simply dissolve it by warming. DNA-ExitusPlus™ is not heat sensitive-quite the contrary: elevated temperatures speed up and improve the nucleic acid destroying activity.

RNase-ExitusPlus™ and DNA-ExitusPlus™ were prepared for Biological Industries by Applichem GmbH.

EZ-DNA Genomic DNA Isolation Kit

Product Name	Catalogue No.	Unit Size
EZ-DNA Genomic DNA Isolation Kit	20-600-50	50ml

EZ-DNA is a non-organic and ready to use reagent for the isolation of genomic DNA from samples of human, animal, plant, yeast, bacterial and viral origin. EZ-DNA is based on disruption of cells in guanidine- detergent lysing solution that hydrolyzes RNA and allows the selective precipitation of DNA from a cell lysate with ethanol. Following an ethanol wash, DNA is solubilized in water or 8 mM NaOH. There is no phenol in EZ-DNA. The protocol is fast and permits isolation of genomic DNA from a large number of samples of small or large volumes. The procedure can be completed in 10-30 minutes with DNA recovery of 70-100%. The isolated DNA can be used, without additional purification, for southern analysis, dot blot hybrization, molecular cloning, RFLP, PCR and other molecular biology and biotechnology applications.

Kit reagents
50ml solution containing Guanidinium Isothiocyanate

Storage
EZ-DNA should be stored at room temperature. Storing at lower temperatures will cause the Guanidine Isothiocyanate to come out of the solution. If the reagent is warmed, the Guanidine Isothiocyanate should resolubilize instantly.

Assessing yield of genomic DNA
The yield of genomic DNA will vary depending on the tissue or cells from which it is obtained.

Tissue/sample type	Amount of starting material	Yield of total RNA
Liver	1mg	3-4µg
Kidney	1mg	3-4µg
Skeletal muscle	1mg	2-3µg
Brain	1mg	2-3µg
Placenta	1mg	2-3µg
Human cells	10^6 cells	5-7µg
Rat cells	10^6 cells	5-7µg
Mouse cells	10^6 cells	5-7µg
Lung	1mg	3-5µg
Heart	1mg	2-3µg
Plant leaf	1gr	20-200µg
Whole blood	1ml	20-40µg
Sf9 cells	10^7 cells	170-180µg
E.coli cells	10^9 cells	30-40µg
Mouse tail	1mg	0.4-3µg

RBC Lysis Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
RBC Lysis Solution (for use with EZ-RNA and EZ-DNA Kits for whole blood)	01-888-1B	100ml	2-8ºC

Red Blood Cells (RBC) Lysis Solution is intended for use in isolation of leukocytes from whole blood. RBC Lysis Solution selectively lyses the erythrocytes leaving the leukocytes. The resulting white blood cells can be readily lysed and processed when isolating nucleic acid with EZ-RNA, EZ-DNA or any other method for nucleic acid isolation from whole blood.

EZ-Plant

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ Plant (for use with 20-600-50)	01-893-1D	10 ml	2-8ºC

For use with EZ-DNA Kit to assist with DNA isolation from plant tissues containing polyphenolics and polysaccharides.
The EZ-Plant solution contains a high molecular weight polymer, Polyvinylpyrrolidone (PVP), which binds the reactive polyphenolic and polysaccharide contaminants present in plant tissues.

EZ-Blood

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ Blood (for use with 20-600-50)	01-894-1B	100 ml	2-8ºC

For use with EZ-DNA kit to assist with DNA isolation from frozen or fresh blood samples.
EZ Blood buffer solution is intended for the isolation of genomic DNA from frozen or fresh blood. The procedure includes a nuclear isolation step prior to DNA extraction. Work time required is 10 minutes.

EZ-ECL Enhanced Chemiluminescence Detection Kit for HRP

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ-ECL Kit	20-500-120	1200cm^2 (120ml)	2-8ºC

Chemiluminescence Detection kit for HRP is a complete kit with ready-to-use reagents for Enhanced chemiluminescent detection of immobilized proteins (Western blotting) or immobilized nucleic acids (Southern or Northern blotting), conjugated with HRP directly or indirectly. The use of enhanced chemiluminescence was introduced by Thorpe and Kricka. In the presence of hydrogen peroxide (H2O2), Horseradish peroxidase (HRP) catalyzes the oxidation of cyclic diacylhydrazides, such as luminol.
Immediately following the oxidation, the luminol is in an excited state (intermediate reaction product), which decays to the ground state by emitting light. Strong enhancement of the light emission is produced by enhancers, such as phenolic compounds. Using this method, it is possible to detect membrane immobilized specific antigens, or sequences of nucleic acids, labeled directly with HRP or indirectly with HRP-labeled antibodies/ streptavidin.

Advantages
- High sensitivity non-radioactive detection system.
- Stable hard copy results on film.
- Only small amounts of antibody required.
- Detection may be achieved in short exposure times (minutes).
- High resolution.

Figure 2: Principles of Protein Detection Procedure

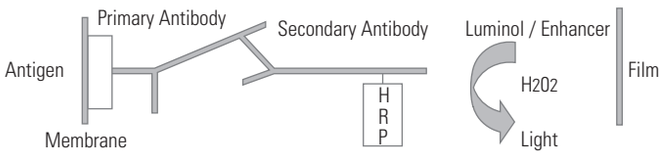
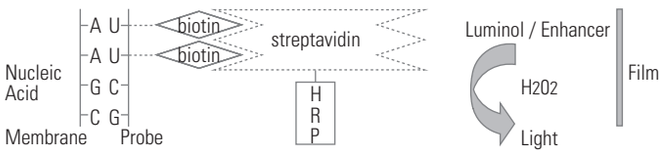
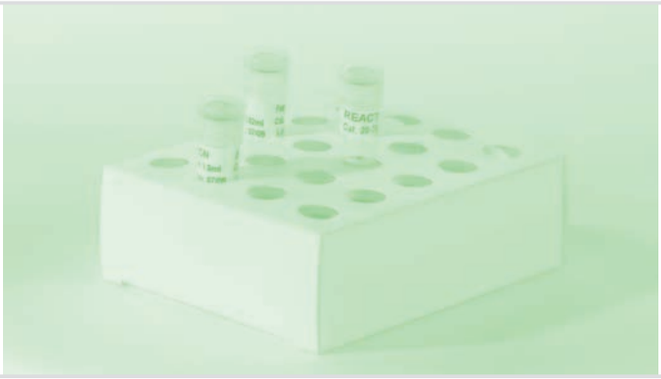


Figure 3: Principles of Nucleic Acid Detection Procedure





Kit Reagents
- Solution A, 60ml, contains: luminol and enhancer
- Solution B, 60ml, contains: stable peroxide solution

EZ-Hybridization Solution

Product Name	Catalogue No.	Unit Size
EZ-Hybridization Solution	01-889-1B	100ml

EZ-Hybridization Solution enables shorter hybridization times and decreases backgrounds. Therefore, low-copy RNA species on Northern blots and single-copy genes on Southern blots can be detected within 1-2 hours of hybridization using radioactively or non-radioactively labeled probes and for both cDNA and oligonucleotide probes.

Storage
- Room temperature
- For long term storage, store at 2-8°C

EZ-Block

Product Name	Catalogue No.	Unit Size
EZ-Block (Blocking reagent for hybridization reactions and Western Blots)	41-805-10	10gr

EZ-Block is used in hybridization and detection procedures using nonradioactive nucleic acid probes, and for Western Blots.
When immunoassays and hybridization assays, such as dot blots, Western blots, Southern blots, or Northern blots are performed, there is nonspecific binding resulting in high background. In order to reduce the nonspecific binding, EZ- Block reagent is used to "block" unbound sites left after immobilization of the specific protein or after the hybridization with nonradioactive probe. EZ-Block improves sensitivity and reduces background.

Storage
Room Temperature

EZ-First Strand cDNA Synthesis Kit for RT-PCR

Product Name	Catalogue No.	Unit Size
EZ-First Strand cDNA Isolation Kit, for RT-PCR	20-800-50	50 reactions

Premixed solutions for the synthesis of single-stranded cDNA from RNA for use as a PCR template.

The Polymerase Chain Reaction (PCR) is a powerful technique for rapid amplification of genes. In addition to amplifying genomic DNA template, PCR can also be used to amplify cDNA reverse transcribed from RNA to analyze gene expression. Using the EZ-First Strand cDNA Synthesis Kit, RNA is reverse transcribed into single-stranded cDNA. The reverse transcriptase (RT) enzyme synthesizes the new cDNA strand at a site determined by the type of primer used: Oligo(dT) primer, random primer or a sequence-specific primer. The first strand cDNA can then be used as a template for PCR.
The reaction mix solution supplied contains buffer, site-directed mutant of MMLV reverse transcriptase and RNase inhibitor sufficient for 50 reactions.
The other ready to use solution contains DTT, oligo dT primer, random primer, control RNA, control primers mix for PCR and DEPC-treated water.

Kit Reagents

1	RT Reaction Mix Contains: Reverse transcriptase, RNase inhibitor and dNTP's in buffer solution	400µl
2	DTT Solution, 100mM	100µl
3	Oligo(dT)20 Primer, 40µM	50µl
4	Random Hexamer Primer, 40µM	50µl
5	Control RNA Human, Total RNA, 1µg/µl	25µl
6	Primer Mix Human G3PDH amplimers, 10µM each	50 µl
7	DEPC-Treated Water	1.5ml

Storage
- The control RNA should be stored at -70°C.
- The other premixed solutions should be stored at -20°C.

Quick-Load

Product Name	Catalogue No.	Unit Size	Storage Temp.
Quick Load 5X Conc. (PCR Loading Solution)	01-892-1H	5ml	2-8ºC

A PCR loading solution for the direct loading of reaction products onto agarose gel.

Advantages

Use of Quick Load, which is a 5x loading dye solution containing a PCR compatible dye and an inert densifying agent, has all of the following advantages:

- Samples can be visualized while loading.
- PCR products can be loaded onto agarose gel immediately following amplification.
- Red dye serves as a marker dye during gel electrophoresis.
- Saves time.
- Easy to use.
- Ideal for 96-well PCR.
- Non-toxic ingredients.

DEPC-Treated Water

Product Name	Catalogue No.	Unit Size
DEPC-Treated Water	01-852-1A	500ml

Random Primer DNA Labeling Mix

Product Name	Catalogue No.	Unit Size
Random Primer DNA Labeling Mix	20-101-25	25 assays

Premixed solution for the labeling of DNA with radiolabeled dCTP using random sequence oligonucleotides.

The use of a "random primed" DNA sequence to prime DNA synthesis was originally introduced by Feinberg and Vogelstein. The method is based on the hybridization of oligonucleotides of all possible sequences to the denatured template DNA to be labeled. The complementary DNA strand is synthesized by a "Klenow" fragment of DNA Polymerase I, using the random oligonucleotides as primers. By substituting a radiolabeled nucleotide for a non-radioactive equivalent in the reaction mixture, the newly synthesized complementary DNA is made radioactive.

The labeling mix system is a specially developed reaction mixture for enhanced convenience and performance. The reaction mixture contains random oligonucleotides, a Klenow fragment of DNA Polymerase I, dATP, dGTP, dTTP and a reaction buffer concentrate. The DNA labeling mix allows the labeling of the template DNA to a specific activity of $2x10^9$ dpm/µg after only 10 minutes of incubation. This rapid labeling is accomplished with the use of the Klenow fragment, which lacks 5'-3' exonuclease activity, and by the use of nonamer primers giving more efficient priming from the template at 37°C. The labeling mix method enables the labeling of small amounts of DNA (10-20ng), such as restriction fragments isolated from gels. Fragments can be labeled directly in low melting temperature agarose gel slices. The labeled probes are used in various hybridization techniques, such as Southern and Northern blots, in-situ hybridization and screening of gene libraries.

Kit reagents

1 vial containing 100µl DNA labeling mixture. Each vial is sufficient for 25 labeling assays.

Storage

The premixed solution should be stored at -20°C. Avoid repeated changes in the solution temperature.

DNA Isolation Kit

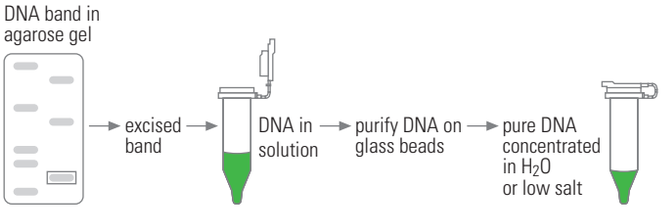
Product Name	Catalogue No.	Unit Size
DNA Isolation Kit	20-200-300	150-300 isolations

The DNA isolation kit provides a convenient method for extracting DNA from agarose gels, isolating plasmid DNA from mini-preps, as well as for concentration of DNA without ethanol precipitation.

Kit reagents
- Glass powder suspension, 1.5ml
- Sodium Iodide solution (6M), 120ml
- Concentrated wash solution, 25ml

Storage
- Sodium Iodide solution: store at 2-8°C, protect from light.
- Concentrated wash solution: store at -20°C in a glass bottle.

Figure 4: Purifying DNA from TAE agarose gel



Water Saturated Phenol

Product Name	Catalogue No.	Unit Size
Water Saturated Phenol pH recovery buffer for pH 7.9 included For use in RNA/DNA extraction	01-860-1L	250ml

Biological Industries Phenol is a liquid phase water-saturated phenol. It has an acid pH value, which allows direct use for RNA extraction.
The water-saturated phenol does not contain any additive or antioxidant and it is packed under argon.
For DNA extraction, the pH of the water-saturated phenol has to be adjusted to pH 7.9 with the pH recovery buffer solution supplied.

Procedure: pH adjustment to 7.9
1. Add the 50ml pH Recovery Buffer Solution directly to the 250ml water saturated phenol.
2. Mix thoroughly and let the phases separate.
3. Use the neutralized phenol under the aqueous phase.

Storage
Water-saturated phenol should be stored at 2-8°C. If necessary, the phenol may be kept frozen at -20°C for long period.
Protect the phenol from light.

Ultra Pure Water (DNase and RNase-free)

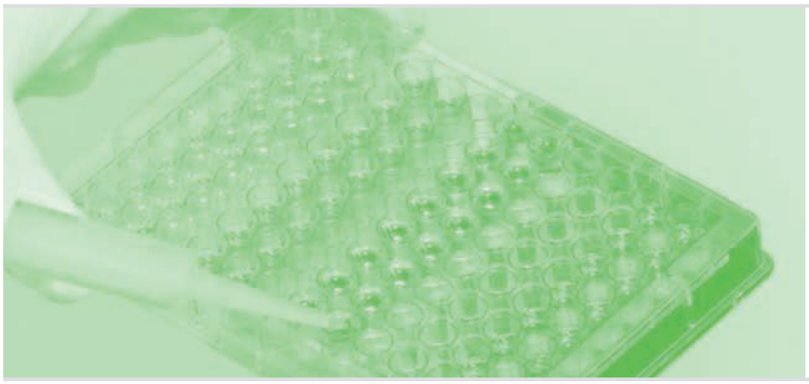
Product Name	Catalogue No.	Unit Size	Storage Temp.
Ultra Pure Water (DNase and RNase-free)	01-866-1A	500ml	AMB
	01-866-1B	100ml	AMB

TAE Buffer 50X Conc.

Product Name	Catalogue No.	Unit Size	Storage Temp.
TAE Buffer 50X Conc.	01-870-1A	500ml	AMB

TBE Buffer 5X Conc.

Product Name	Catalogue No.	Unit Size	Storage Temp.
TBE Buffer 5X Conc.	01-871-1A	500ml	AMB



Acrylamide / Bis-Acrylamide (T=40%)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Acrylamide/Bis-Acrylamide (19:1 ratio) (T=40%)	01-872-1A	500ml	2-8ºC
Acrylamide/Bis-Acrylamide (29:1 ratio) (T=40%)	01-874-1A	500ml	2-8ºC
Acrylamide/Bis-Acrylamide (37.5:1 ratio) (T=40%)	01-876-1A	500ml	2-8ºC

Polyacrylamide Gels

Polyacrylamide is a commonly used electrophoresis matrix for size separation of proteins and nucleic acids. The gel matrix is formed by free radical polymerization of acrylamide and a comonomer crosslinker (bis-acrylamide).

The gel pore size is determined by two parameters:

- Total monomer concentration (%T)
- The weight percentage of crosslinker (%C)

$$\%T = \frac{\text{gram (acrylamide + bis-acrylamide)}}{\text{Total volume (ml)}} \text{X100}$$

$$\%T = \frac{\text{gram (bis-acrylamide)}}{\text{gram (acrylamide + bis-acrylamide)}} \text{X100}$$

Gel with T=20% is prepared with 20% of acrylamide and bis-acrylamide. As %T is higher, the pore size of the gels are smaller.
Gel with T=20%, C=5% is prepared with 20% of acrylamide and bis-acrylamide and the weight percentage of the bis-acrylamide is 5% from the total weight of the acrylamide and bisacrylamide.
Biological Industries' acrylamide and bis-acrylamide solutions are available in 3 different crosslinker ratios: 19:1, 29:1 and 37.5 :1.

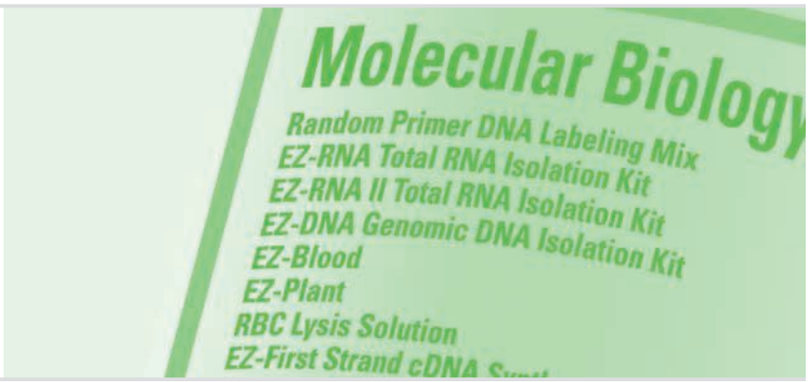
Standard protocol for SDS-PAGE gel

The solutions (10ml resolving gel and 10ml stacking gel) may be used for the preparation of two gels with the size of 100mmx80mmx1.4mm.

	Resolving gel 10ml			Stacking gel 10ml
Final concentration of gel	7%	10%	12.50%	5%
Acrylamide-bis solution (T=40%)	1.75ml	2.5ml	3.13ml	1.25ml
Resolving buffer, 4x (w/o SDS)	2.5ml	2.5ml	2.5ml	---
Stacking buffer, 4x (w/o SDS)	---	---	---	2.5ml
Distilled water	5.65ml	4.9ml	4.27ml	6.15ml
SDS, 10% solution	0.1ml	0.1ml	0.1ml	0.1ml
TEMED	0.015ml	0.015ml	0.015ml	0.015ml
Ammonium persulphate, 10% solution	0.03ml	0.03ml	0.03ml	0.03ml

SDS Solution (10%)

Product Name	Catalogue No.	Unit Size	Storage Temp.
SDS Solution (10%)	01-890-1B	100ml	AMB



Product Name	Catalogue No.	Unit Size	Storage Temp.
Kits for Molecular Biology			
Random Primer DNA Labeling Mix	20-101-25	25 assays	-20ºC
DNA Isolation Kit	20-200-300	150-300 isolations	2-8ºC
EZ-RNA Total RNA Isolation Kit	20-400-100	100ml	2-8ºC
EZ-RNA II Total RNA Isolation Kit Without Chloroform, with BCP	20-410-100	100ml	2-8ºC
EZ-ECL Kit	20-500-120	1200cm^2 (120ml)	2-8ºC
EZ-DNA Genomic DNA Isolation Kit	20-600-50	50ml	AMB
EZ-First Strand cDNA Isolation Kit, for RT-PCR	20-800-50	50 reactions	-20ºC
Auxiliary products			
DEPC-Treated Water	01-852-1A	500ml	AMB
Water Saturated Phenol, PH recovery buffer for PH 7.9 included For use in RNA/DNA extraction	01-860-1L	250ml	2-8ºC
Ultra Pure Water (DNase and RNase-free)	01-866-1A	500ml	AMB
	01-866-1B	100ml	AMB
TAE Buffer 50X Conc.	01-870-1A	500ml	AMB
TBE Buffer 5X Conc.	01-871-1A	500ml	AMB
Acrylamide/Bis-Acrylamide (19:1 ratio) (T=40%)	01-872-1A	500ml	2-8ºC
Acrylamide/Bis-Acrylamide (29:1 ratio) (T=40%)	01-874-1A	500ml	2-8ºC
Acrylamide/Bis-Acrylamide (37.5:1 ratio) (T=40%)	01-876-1A	500ml	2-8ºC
RBC Lysis Solution	01-888-1B	100ml	2-8ºC
EZ-Hybridization Solution	01-889-1B	100ml	AMB
SDS Solution (10%)	01-890-1B	100ml	AMB
RNA Save	01-891-1A	500ml	2-8ºC
	01-891-1B	100ml	2-8ºC
	01-891-1C	20ml	2-8ºC
Quick Load 5X Conc. (PCR Loading Solution)	01-892-1H	5ml	2-8ºC
EZ Plant	01-893-1D	10 ml	2-8ºC
EZ Blood	01-894-1B	100 ml	2-8ºC
EZ-Block	41-805-10	10gr	AMB
Rnase-ExitusPlus™	01-897-1B	100ml	AMB
	01-897-1L	250ml	AMB
DNA-ExitusPlus™	01-898-1B	100ml	AMB
	01-898-1L	250ml	AMB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1,00
0,75
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0,25

CYTOKINES & GROWTH FACTORS

17

CYTOKINES & GROWTH FACTORS

Cytokines are a large and diverse family of polypeptide regulators, signaling proteins and glycoproteins, which- like hormones and neurotransmitters- are critical to the development and functioning of both innate and adaptive immune response, as well as to other body systems. While hormones are secreted from specific organs to the blood and neurotransmitters are related to neural activity, cytokines are more of a diverse category or class of compounds in terms of origin and function.

These signaling molecules, historically known as immodulating agents (e.g. interferons, interleukins) like neurotransmitters, hormones and growth factors, function as mediators, directly and indirectly, in cellular communication by triggering specific cellular reactions or responses in target tissues and organs throughout many areas of the body, or to distinct, specific cells. Neurotransmitters act in a similar fashion when released at an axon terminal of a neuron that eventually locks on to a specific receptor site or target impacting another neuron, muscle or gland cell. Growth factors like cytokines are secreted by a variety of cells and bind to or lock on to specific high-affinity cell-surface target receptors, thereby eliciting similar or more specific biochemical changes through a cascade of events. Whereas the effects of cytokines include autocrine, endocrine and paracrine activity relative to target organ or cell-receptor site, growth factors not only elicit a cascade of events by participating in the regulation of cell growth and differentiation, but also by promoting replication and similar complex processes.

The cytokine family includes a variety of colony and growth-stimulating factors, interferons, interleukins, chemokines and other molecules that exhibit pleiotropic effects and considerable redundancy. They may act on the cellular level such as in cell differentiation, through tissue development, homeostasis, and in certain developmental processes, during embryogenesis. Due to the fact that they are characterized by such general effects and hence are often difficult to objectively characterize, such aforementioned distinctions, allowing for exceptions, are for the most part null and void. Nevertheless, this much is known about them. These distinct effects are multi-fold and depend upon, inter alia:

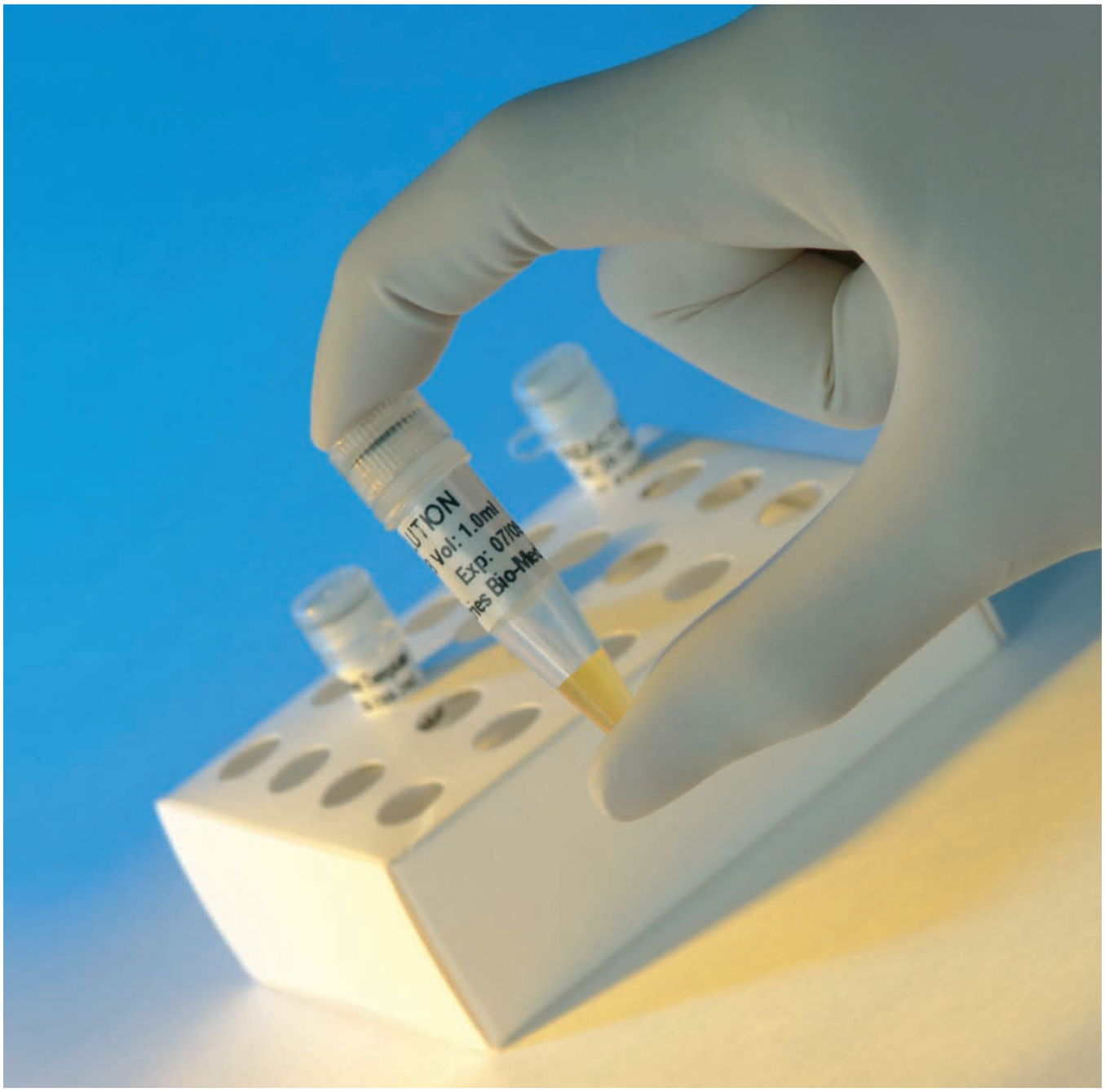
- The specific cytokine per se.
- The abundance or concentration of the cytokine relative to its location or target receptor.
- The complementary high-affinity receptor site or sites (presence and abundance).
- The quality of downstream signals triggered by the interaction of receptor binding activity.

For example, a variety of cytokines and growth factors influence the invasive properties of extravillous trophoblast and surrounding cells.
Of these, Epidermal Growth Factor (EGF), Insulin-like Growth Factor II (IGF-II), Transforming Growth Factor ß (TGF-ß,) and Granulocyte-Macrophage Colony Stimulating Factor (GMCSF) and others have been extensively studied in human implantation. Over fifty different proteins that function as growth factors have been isolated and identified with many more possibilities on the horizon. While some growth factors affect a broad range of cell types (e.g. EGF, PDGF) others are much more specific. Nerve Growth Factor (NGF), for instance, promotes the growth of certain classes of neurons, while erythropoietin stimulates red blood cell precursors triggering cell division. Most mammalian cells in culture need a combination of different growth factors and/or cytokines to impact the various controls that inhibit cell division or to elicit other more receptor-specific and distinct effects.

Biological Industries' Cytokines and Growth Factors are manufactured under strict and vigorous Quality Assurance Protocols and comply with verifiable product specifications. They are of the highest quality, exhibit high levels of bioactivity performance and are competitively priced.

Cytokines
Adiponectin (10 products)
Angiopoietin (4 products)
Apoliprotein (8 products)
B-cell Activating Factor (3 products)
Beta Defensin (4 products)
Bone Morphogenetic Protein (8 products)
B type Natriuretic Peptide (3 products)
Endoglin (3 products)
Flt-3 Ligand (3 products)
Hedgehog Protein (2 products)
Interleukin (92 products)
Interferon (19 products)
Resistin (9 products)
Thrombopoietin (3 products)
Trefoil Factor (5 products)
Tumor Necrosis Factor (11 products)
Visfatin (3 products)
Other (13 products)

Growth Factors
Colony Stimulating Factor (14 products)
CTGF (3 products)
Epidermal Growth Factor (6 products)
Erythropoietin (2 products)
Fibroblast Growth Factor (21 products)
Galectin (2 products)
Growth Hormone (21 products)
Hepatocyte Growth Factor (3 products)
Insulin-Like Growth Factor (10 products)
Insulin (3 products)
Keratinocyte Growth Factor (2 products)
Leptin (25 products)
Macrophage Migration Inhibitory Factor (4 products)
Myostatin (3 products)
Noggin (2 products)
PDGF (9 products)
Placental Lactogen (3 products)
Prolactin (14 products)
Stem Cell Factor (4 products)
Transforming Growth Factor (4 products)
VEGF (18 products)
Other (18 products)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HUMAN SERUM & BLOOD PRODUCTS

18

HUMAN SERUM & BLOOD PRODUCTS

Biological Industries' Pre-Screened and Pre-tested human serum and blood products undergo the most stringent and rigorous Quality Control/Assurance Standards and Protocols testing all raw materials and finished products in order to meet the demands of international markets and ensure high quality and consistency.
All our human serum and blood products meet approved compliance validation and specifications prior to use and/or release of the final product to the end-user.
All our human serum and blood products undergo a methodical and comprehensive battery of physico-chemical, microbiological and biological performance testing procedures. Each batch is traceable, well-documented from source of origin through the thorough and systematic Quality Control process. All documentation and certification are available upon request.

Selecting the appropriate Human Serum Proteins depends upon at least four key factors:

- The cell line.
- The type of culture system.
- The chemical composition of the basal medium.
- The experience of the researcher.

Human Serum Albumin (HSA)

Product Name	Catalogue No.	Unit Size	Storage Temp.
Bio-Pure Human Serum Albumin (HSA Solution, 10%) Optimized for Human Embryonic Stem Cells (hESC)	05-720-1B	100ml	-20ºC
	05-720-1E	50ml	-20ºC

HSA is a medium supplement that is a highly soluble osmolytic protein with a high molecular weight. It was specifically developed to support and maintain cell development, growth, health and productivity in most cell culture media, and especially cell membrane stability. The primary function of HSA is not only its unique demonstrative capability of binding anionic, cationic and neutral molecules, but it also has the proclivity of sequestering and stabilizing a wide array of ions and other small molecules.
HSA complies with the specifications of the manufacturer and the requirements stipulated by FDA approved tests.
All individual donations of the plasma and the corresponding plasma pool, has been tested for Hepatitis B Surface Antigen (HBsAg), Anti (Human Immunodeficiency Virus) HIV-I and II and anti-HCV and found to be negative.

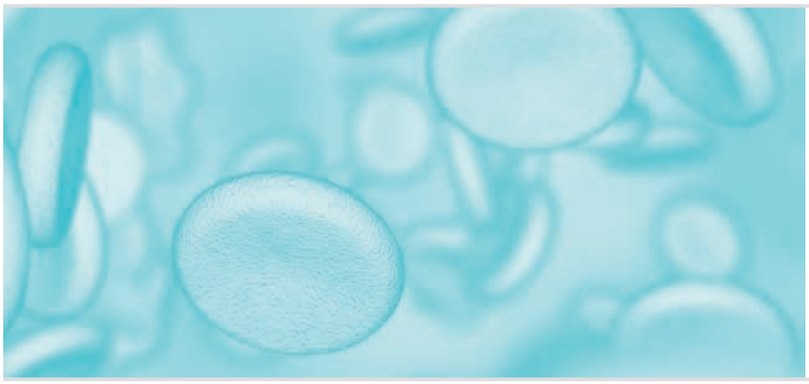
Transferrin, Human, Substantially Iron-Free (APO) & Transferrin, Human, Iron-Saturated (HOLO)

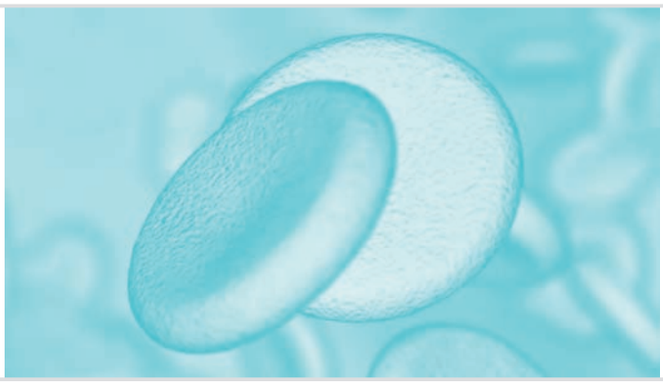
Product Name	Catalogue No.	Unit Size	Storage Temp.
Transferrin, Human, Substantially Iron-Free (APO)	41-951-100	100mg	2-8ºC
	41-951-500	500mg	2-8ºC
Transferrin, Human, Iron-Saturated (HOLO)	41-952-100	100mg	2-8ºC
	41-952-500	500mg	2-8ºC

Transferrin is an important constituent in growth medium. It is a glycoprotein, but also known more specifically, as an iron-storage protein, found in mammalian serum (i.e. a blood plasma protein). Transferrin receptors, on the cell surface of actively growing cells, bind transferrin for iron transport to and from cells. In humans, it is still the most dynamically important iron pool relative to the total iron throughout the body. Nevertheless, research has shown that the majority of circulating iron-bound transferrin is transported to the bone marrow and incorporated into newly formed red blood cells or erythrocytes. The other primary storage depots for stored iron are the liver and spleen. The sum of all iron-binding sites on transferrin constitutes the total iron-binding capacity or TIBC of plasma. When iron-free, transferrin is known as apo-Transferrin and when iron saturated it is called holo-Transferrin. Traditionally, transferrin has entered the cell culture domain as a component of serum and is not a routine component of most commercially produced basal media. However, it has gained popularity and it is often added to classical basal media for the delivery of iron especially in a serum-free milieu. As a plasma-derived product, it is available for cell culture and diagnostic assays but not for therapeutic use.

Each unit of Biological Industries' transferrin is pasteurized and heated for 10 Hours @60ºC. It is manufactured under GMP conditions from Human Blood Plasma sub Fraction IV-1. It is important to emphasize that it is for research, laboratory or further manufacturing purposes only. It is not intended for human use. It is a USA-sourced and approved product and plasma donors undergo a rigorous selection process as per FDA requirements.

Each unit of plasma and each plasma pool has been tested to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Antibody to (Human Immunodeficiency Virus) HIV-I and II, anti-HCV and Syphilis.





EZ Lympho-Sep™-Lymphocyte Separation Tubes

Ready-to-use
Alternative to the "home made" blood separation tube

Density gradient centrifugation of whole blood on a polysucrose - sodium metrizoate medium is the method of choice for isolation of lymphocytes. The success of the procedure, i.e. the recovery of viable lymphocytes with the lowest proportion of contaminating granulocytes and erythrocytes, depends to a large extent on the careful layering of the blood sample onto the separation medium and the maintenance of a sharp interface between the two solutions prior to centrifugation.
The EZ Lympho-Sep™ system allows the blood sample to be poured directly into the centrifuge tube with no special precautions required to prevent disruption of the polysucrose - sodium metrizoate layer. Thus, a large number of samples may be handled at the same time. The mechanism also reduces the length of centrifugation time required for separation of the lymphocytes.

The heart of the EZ Lympho-Sep™ is a plastic insert that allows the blood sample to be poured directly into the tube alleviating the need for slow and careful addition of the blood. Secondly, a one-way feature of the insert allows passage of materials during the centrifugation step but prevents the flow of the separation medium during shipping. After centrifugation, the upper lymphocyte-containing fraction may be poured off without risk of contamination from the erythrocytes, which are trapped under the insert.

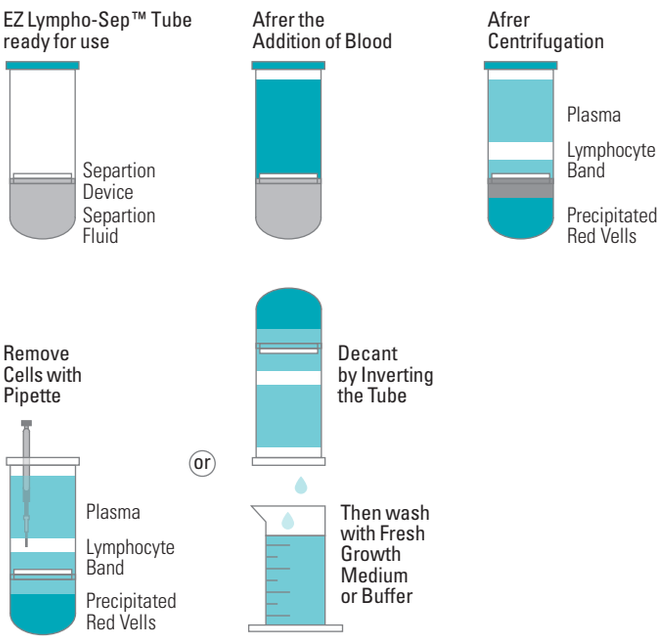
EZ Lympho-Sep™ are ready-to-use sterile tubes with the separation medium already in place, and the one-way valve opening during centrifugation.
We offer four different sizes:

Catalogue No.	Separation Medium	Centrifuge Tube	Packaging
01-899-U02	2ml	15ml	30 Tubes/Box
01-899-U04	3ml	15ml	30 Tubes/Box
01-899-U10	10ml	50ml	18 Tubes/Box
01-899-U16	15ml	50ml	18 Tubes/Box

We can also supply empty tubes for customers who have their own media.

Features
- Ready-to-use, sterile.
- Safe method, minimum contact with biological fluids.
- Time saver, quick and easy sample filling.
- Maximum yield of viable mononuclear cells.
- A large number of samples may be handled at the same time.

Figure 1: The EZ Lympho-Sep™ system



Related products

Product Name	Catalogue No.	Unit Size	Storage Temp.
EZ-DNA Genomic DNA Isolation Kit *	20-600-50	50ml	AMB
EZ Blood *	01-894-1B	100 ml	2-8°C
RBC Lysis Solution *	01-888-1B	100ml	2-8°C
Phytohemagglutinin-M (PHA-M), Lyophilized **	12-006-1H	5ml	2-8°C

* See chapter 16 - Molecular Biology
** See chapter 11 - Human Cytogenetics

APPENDIXES

Formulations
Certifications
Worldwide Distributors
Representation of Companies in the Domestic Market
Alphabetical Index by Product Name
Index by Catalogue Number

19

FORMULATIONS

Basal Medium-Eagle (BME)[1]

Product Catalogue No. Component	BME Earle's Salt Base 01-015-1* mg/liter	BME 10x Earle's Salt Base 01-015-5*+ mg/liter
CaCl2·2H2O	264.92	2649.2
KCl	400.0	4000.0
KH_2PO_4	-	-
$MgCl_2·6H_2O$	-	-
$MgSO_4·7H_2O$	200.0	2000.0
NaCl	6800.0	68000.0
$NaHCO_3$	2200.0	-
$NaH_2PO_4·H_2O$	140.0	1400.0
$Na_2HPO_4·7H_2O$	-	-
D-GLUCOSE	1000.0	10000.0
PHENOL RED	10.0	100.0
L-ARGININE HCl	21.0	210.0
L-CYSTINE	12.0	120.0
L-GLUTAMINE	292.0*	2920.0*
L-HISTIDINE HCl·H_2O	10.5	105.0
L-ISOLEUCINE	26.0	260.0
L-LEUCINE	26.0	260.0
L-LYSINE HCl	36.5	365.3
L-METHIONINE	7.5	75.0
L-PHENYLALANINE	16.5	165.0
L-THREONINE	24.0	240.0
L-TRYPTOPHAN	4.0	40.0
L-TYROSINE	18.0	180.0
L-VALINE	23.5	235.0
D-BIOTIN	1.0	10.0
D-CALCIUM PANTOTHENATE	1.0	10.0
CHOLINE CHLORIDE	1.0	10.0
FOLIC ACID	1.0	10.0
i-INOSITOL	2.0	20.0
NICOTINAMIDE	1.0	10.0
PYRIDOXAL HCl	1.0	10.0
RIBOFLAVIN	0.1	1.0
THIAMINE HCl	1.0	10.0

* This preparation is without L-Glutamine.
\+ All media concentrates 10X are prepared without Sodium Bicarbonate.
(1) Eagle, H., Exptl. Soc. Biol. Med., 89:362, (1955).

Modified Eagle's Minimum Essential Medium

Dulbecco's Modified Eagle's Media[1] (DMEM) Iscove's Modified Dulbecco's Media[2] (IMDM)

Product Catalogue No. Component	DMEM 1X Low Gluc. 01-050-1* mg/liter	DMEM 5X Low Gluc. 01-050-4*+ mg/liter	DMEM 1X High Gluc. 01-055-1* mg/liter	DMEM 5X High Gluc. 01-055-4*+ mg/liter	ISCOVE'S 1X 01-058-1 mg/liter
$CaCl_2·2H2O$	264.92	1324.6	264.92	1324.6	218.6
$Fe(NO_3)_3·9H2O$	0.1	0.5	0.1	0.5	-
KCl	400.0	2000.0	400.0	2000.0	330.0
KNO_3	-	-	-	-	0.076
$MgSO_4·7H_2O$	200.0	1000.0	200.0	1000.0	200.0
NaCl	6400.0	32000.0	6400.0	32000.0	4505.0
$NaHCO_3$	3700.0	-	3700.0	-	3024.0
$NaH_2PO_4·H_2O$	125.0	625.0	125.0	625.0	125.0
$NaSeO_3·5H_2O$	-	-	-	-	0.0173
D-GLUCOSE	1000.0	5000.0	4500.0	22500.0	4500.0
PHENOL RED	15.0	75.0	15.0	75.0	15.0
SODIUM PYRUVATE	110.0	550.0	-	-	110.0
HEPES	-	-	-	-	5958.0
L-ALANINE	-	-	-	-	25.0
L-ARGININE HCl	84.0	420.0	84.0	420.0	84.0
L-ASPARAGINE·H_2O	-	-	-	-	28.4
L-ASPARTIC ACID	-	-	-	-	30.0
L-CYSTINE	48.0	240.0	48.0	240.0	70.0
L-GLUTAMIC ACID	-	-	-	-	75.0
L-GLUTAMINE	584.0*	2920.0*	584.0*	2920.0*	584.0
GLYCINE	30.0	150.0	30.0	150.0	30.0
L-HISTIDINE HCl·H_2O	42.0	210.0	42.0	210.0	42.0
L-ISOLEUCINE	105.0	525.0	105.0	525.0	105.0
L-LEUCINE	105.0	525.0	105.0	525.0	105.0
L-LYSINE HCl	146.0	730.0	146.0	730.0	146.0
L-METHIONINE	30.0	150.0	30.0	150.0	30.0
L-PHENYLALANINE	66.0	330.0	66.0	330.0	66.0
L-PROLINE	-	-	-	-	40.0
L-SERINE	42.0	210.0	42.0	210.0	42.0
L-THREONINE	95.0	475.0	95.0	475.0	95.0
L-TRYPTOPHAN	16.0	80.0	16.0	80.0	16.0
L-TYROSINE	72.0	360.0	72.0	360.0	72.0
L-VALINE	94.0	470.0	94.0	470.0	94.0
D-BIOTIN	-	-	-	-	0.013

Product Catalogue No. Component	DMEM 1X Low Gluc. 01-050-1* mg/liter	DMEM 5X Low Gluc. 01-050-4*+ mg/liter	DMEM 1X High Gluc. 01-055-1* mg/liter	DMEM 5X High Gluc. 01-055-4*+ mg/liter	ISCOVE'S 1X 01-058-1 mg/liter
D-CALCIUM PANTOTHENATE	4.0	20.0	4.0	20.0	4.0
CHOLINE CHLORIDE	4.0	20.0	4.0	20.0	4.0
FOLIC ACID	4.0	20.0	4.0	20.0	4.0
i-INOSITOL	7.2	36.0	7.2	36.0	7.2
NICOTINAMIDE	4.0	20.0	4.0	20.0	4.0
PYRIDOXAL HCl	4.0	20.0	4.0	20.0	4.0
RIBOFLAVIN	0.4	2.0	0.4	2.0	0.4
THIAMINE HCl	4.0	20.0	4.0	20.0	4.0
VITAMIN B_{12}	-	-	-	-	0.013

* This preparation is without L-Glutamine.
\+ All media concentrates 5X are prepared without Sodium Bicarbonate.
(1) Dulbecco, R. and Freeman, G., Virology, 8:396, (1959).
Smith, J.D. et al, Virology, 12:185, (1960).
TCA Standards Committee, In Vitro, Vol. 6/2:93, (1970).
(2) Iscove, N.N. and Melchers, F., Jour. of Exp. Med., Vol. 147:923.

Ribonucleosides and Deoxyribonucleosides for MEM Alpha (500x Concentrate)

Catalogue No. 01-343-1	mg/ml
ADENOSINE	5.0
CYTIDINE	5.0
GUANOSINE	5.0
URIDINE	5.0
2'-DEOXYADENOSINE	5.0
2'-DEOXYCYTIDINE HCl	5.5
2'-DEOXYGUANOSINE	5.0
2'-DEOXYTHYMIDINE	5.0

Minimum Essential Medium Alpha

Catalogue No. 01-042-1 Component	mg/liter
$CaCl_2$·$2H_2O$	265.0
$Fe(NO3)_3$·$9H_2O$	-
KCl	400.0
$MgSO_4$·$7H_2O$	200.0
NaCl	6800.0
$NaHCO_3$	2200.0
NaH_2PO_4·H_2O	140.0
D-GLUCOSE	1000.0
PHENOL RED	10.0
SODIUM PYRUVATE	110.0
LIPOIC ACID	0.2
L-ALANINE	25.0
L-ARGININE HCl	127.0
L-ASPARAGINE·H2O	50.0
L-ASPARTIC ACID	30.0
L-CYSTINE	24.0
L-CYSTEINE HCl·H2O	100.0
L-GLUTAMIC ACID	75.0
L-GLUTAMINE	292.0
GLYCINE	50.0
L-HISTIDINE HCl·H2O	42.0
L-ISOLEUCINE	52.5
L-LEUCINE	52.4

Catalogue No. 01-042-1 Component	mg/liter
L-LEUCINE	52.4
L-LYSINE HCl	72.4
L-METHIONINE	15.0
L-PHENYLALANINE	32.0
L-PROLINE	40.0
L-SERINE	25.0
L-THREONINE	48.0
L-TRYPTOPHAN	10.0
L-TYROSINE	36.0
L-VALINE	46.0
L-ASCORBIC ACID	50.0
D-BIOTIN	0.1
D-CALCIUM PANTOTHENATE	1.0
CHOLINE CHLORIDE	1.0
FOLIC ACID	1.0
i-INOSITOL	2.0
NICOTINAMIDE	1.0
PYRIDOXAL HCl	1.0
RIBOFLAVIN	0.1
THIAMINE HCl	1.0
TRYPTOSE PHOSPHATE BROTH	-
VITAMIN B_{12}	1.36

* This preparation is without L-Glutamine.
\+ All media concentrates 10X are prepared without Sodium Bicarbonate.
(1) Nature, New Biology 230:310, (1971).
(2) MacPherson, J. and Stoker, M., Virology, 16:147, (1962).

Roswell Park Memorial Institute Tissue Culture Media Series

Product	RPMI 1640[1]	RPMI 1640[1] 10x	RPMI-1640 With Hepes	McCOY'S 5A[2] Modified
Catalogue No. Component	01-100-1 mg/liter	01-104-5*+ mg/liter	01-106-1 mg/liter	01-075-1 mg/liter
$CaCl_2$	-	-	-	100.0
$Ca(NO3)_2 \cdot 4H O$	100.0	1000.0	100.0	-
KCl	400.0	4000.0	400.0	400.0
$MgSO_4 \cdot 7H_2O$	100.0	1000.0	100.0	200.0
NaCl	6000.0	60000.0	5500.0	6460.0
$NaHCO_3$	2000.0	-	2000.0	2200.0
$Na_2HPO_4 \cdot 7H_2O$	1512.0	15120.0	1512.0	-
$NaH_2PO_4 \cdot H_2O$	-	-	-	580.0
D-GLUCOSE	2000.0	20000.0	2000.0	3000.0
PHENOL RED	5.0	50.0	5.0	5.0
L-ALANINE	-	-	-	13.4
L-ARGININE	200.0	2000.0	200.0	34.9
L-ASPARAGINE-H2O	50.0	500.0	50.0	45.0
L-ASPARTIC ACID	20.0	200.0	20.0	19.9
L-CYSTEINE	-	-	-	31.5
L-CYSTINE	50.0	500.0	50.0	-
L-GLUTAMIC ACID	20.0	200.0	20.0	22.1
L-GLUTAMINE	300.0	3000.0*	300.0	219.2
GLUTATHIONE (Reduced)	1.0	10.0	1.0	0.5
GLYCINE	10.0	100.0	10.0	7.5
L-HISTIDINE	15.0	150.0	15.0	15.25
L-HYDROXYPROLINE	20.0	200.0	20.0	19.7
L-ISOLEUCINE	50.0	500.0	50.0	39.3
L-LEUCINE	50.0	500.0	50.0	39.3
L-LYSINE HCl	40.0	400.0	40.0	36.5
L-METHIONINE	15.0	150.0	15.0	14.9
L-PHENYLALANINE	15.0	150.0	15.0	16.5
L-PROLINE	20.0	200.0	20.0	17.3
L-SERINE	30.0	300.0	30.0	26.3
L-THREONINE	20.0	200.0	20.0	17.9
L-TRYPTOPHAN	5.0	50.0	5.0	3.1
L-TYROSINE	20.0	200.0	20.0	18.1
L-VALINE	20.0	200.0	20.0	17.6
ASCORBIC ACID	-	-	-	0.5
D-BIOTIN	0.2	2.0	0.2	0.2
VITAMIN B_{12}	0.005	0.05	0.005	2.0
D-CALCIUM PANTOTHENATE	0.25	2.5	0.25	0.2
CHOLINE CHLORIDE	3.0	30.0	3.0	5.0
FOLIC ACID	1.0	10.0	1.0	10.0
i-INOSITOL	35.0	350.0	35.0	36.0
NICOTINAMIDE	1.0	10.0	1.0	0.5
NICOTINIC ACID	-	-	-	0.5
p-AMINOBENZOIC ACID	1.0	10.0	1.0	1.0
PYRIDOXAL HCl	-	-	-	0.5
PYRIDOXINE HCl	1.0	10.0	1.0	0.5
RIBOFLAVIN	0.2	2.0	0.2	0.2
THIAMINE HCl	1.0	10.0	1.0	0.2
BACTO PEPTONE	-	-	-	600.0
HEPES	-	-	5958.0	-

Catalogue No. **01-100-1** RPMI-1640[1] with L-Glutamine
Catalogue No. **01-104-5***+ RPMI-1640[1] 10X without L-Glutamine
Catalogue No. **01-107-1** RPMI-1640 DUTCH Modification with L-Glutamine
Catalogue No. **01-075-1** McCOY'S 5A[2] (Modified) with L-Glutamine

* This preparation is without L-Glutamine.
\+ All media concentrates 10X are prepared without Sodium Bicarbonate.
[1] Moore, G., Gerner, R.E. and Franklin H.A., J.A.M.A., 199, (1967).
[2] Iwakata, S. and Grace Jr. J.T., N.Y. State J. Med., 64/18, (1964) (Similar to RPMI-1629 Medium).

Medium M-199

Product Catalogue No. Component	M-199 E Earle's Salts Base 01-080-1 mg/liter	M-199 E Earle's Salts Base 10x 01-080-5+ mg/liter	M-199 H Hanks' Salts Base 01-085-1 mg/liter
$CaCl_2$	200.0	2000.0	140.0
$Fe(NO_3)3{\cdot}9H2O$	0.72	7.2	0.72
KCl	400.0	4000.0	400.0
$KH2PO_4$	-	-	60.0
$MgSO_4.7H_2O$	200.0	2000.0	200.0
NaCl	6800.0	68000.0	8000.0
$NaHCO_3$	2200.0	-	350.0
$NaH_2PO_4{\cdot}H_2O$	140.0	1400.0	-
$Na_2HPO_4{\cdot}7H_2O$	-	-	90.0
D-GLUCOSE	1000.0	10000.0	1000.0
PHENOL RED	20.0	200.0	20.0
ADENINE SULPHATE	10.0	100.0	10.0
ADENOSINE TRIPHOSPHATE Na4	1.0	10.0	1.0
ADENYLIC ACID	0.2	2.0	0.2
CHOLESTEROL	0.2	2.0	0.2
D-2-DEOXYRIBOSE	0.5	5.0	0.5
GLUTATHIONE (Reduced)	0.05	0.5	0.05
GUANINE HCl	0.3	3.0	0.3
HYPOXANTHINE	0.3	3.0	0.3
D-RIBOSE	0.5	5.0	0.5
SODIUM ACETATE	50.0	500.0	50.0
THYMINE	0.3	3.0	0.3
TWEEN 80	20.0	200.0	20.0
URACIL	0.3	3.0	0.3
XANTHINE	0.3	3.0	0.3
L-ALANINE	25.0	250.0	25.0
L-ARGININE HCl	70.0	700.0	70.0
L-ASPARTIC ACID	30.0	300.0	30.0
$L\text{-CYSTEINE HCl}{\cdot}H_2O$	0.11	1.1	0.11
L-CYSTINE	20.0	200.0	20.0
$L\text{-GLUTAMIC ACID}{\cdot}H_2O$	75.0	750.0	75.0
L-GLUTAMINE	100.0	1000.0	100.0
GLYCINE	50.0	500.0	50.0
$L\text{-HISTIDINE HCl}{\cdot}H_2O$	21.88	218.8	21.88
L-HYDROXYPROLINE†	10.0	100.0	10.0
L-ISOLEUCINE	20.0	200.0	20.0
L-LEUCINE	60.0	600.0	60.0

Product Catalogue No. Component	M-199 E Earle's Salts Base 01-080-1 mg/liter	M-199 E Earle's Salts Base 10x 01-080-5+ mg/liter	M-199 H Hanks' Salts Base 01-085-1 mg/liter
L-LYSINE HCl	70.0	700.0	70.0
L-METHIONINE	15.0	150.0	15.0
L-PHENYLALANINE	25.0	250.0	25.0
L-PROLINE	40.0	400.0	40.0
L-SERINE	25.0	250.0	25.0
L-THREONINE	30.0	300.0	30.0
L-TRYPTOPHAN	10.0	100.0	10.0
L-TYROSINE	40.0	400.0	40.0
L-VALINE	25.0	250.0	25.0
ASCORBIC ACID	0.05	0.5	0.05
ALPHA TOCOPHEROL PHOSPHATE Na_2	0.01	0.1	0.01
D-BIOTIN	0.01	0.1	0.01
CALCIFEROL	0.1	1.0	0.1
D-CALCIUM PANTOTHENATE	0.01	0.1	0.01
CHOLINE CHLORIDE	0.5	5.0	0.5
FOLIC ACID	0.01	0.1	0.01
i-INOSITOL	0.05	0.5	0.05
MENADIONE	0.01	0.1	0.01
NIACIN	0.025	0.25	0.025
NIACINAMIDE	0.025	0.25	0.025
p-AMINOBENZOIC ACID	0.05	0.5	0.05
PYRIDOXAL HCl	0.025	0.25	0.025
PYRIDOXINE HCl	0.025	0.25	0.025
RIBOFLAVIN	0.01	0.1	0.01
THIAMINE HCl	0.01	0.1	0.01
VITAMIN A	0.1	1.0	0.1

+ All media concentrates 10X are prepared without Sodium Bicarbonate.
Morgan, Morton and Parker, Proc. Soc. Biol. Med., 73:1, (1950).

Leibovitz L-15 Medium

Catalogue No. 01-115-1 Component	mg/liter
$CaCl_2$	140.0
KCl	400.0
$KH2PO_4$	60.0
$MgCl_2{\cdot}6H_2O$	200.0
$MgSO_4{\cdot}7H_2O$	200.0
NaCl	8000.0
$Na2HPO_4$	190.0
D(+)GALACTOSE	900.0
PHENOL RED	10.0
SODIUM PYRUVATE	550.0
DL-ALPHA ALANINE	450.0
L-ARGININE	500.0
L-ASPARAGINE	250.0
L-CYSTEINE	120.0
L-GLUTAMINE	300.0
GLYCINE	200.0
L-HISTIDINE	250.0
DL-ISOLEUCINE	250.0

Catalogue No. 01-115-1 Component	mg/liter
L-LEUCINE	125.0
L-LYSINE	75.0
DL-METHIONINE	150.0
DL-PHENYLALANINE	250.0
L-SERINE	200.0
DL-THREONINE	600.0
L-TRYPTOPHAN	20.0
L-TYROSINE	300.0
DL-VALINE	200.0
DL-CALCIUM PANTOTHENATE	1.0
CHOLINE CHLORIDE	1.0
FOLIC ACID	1.0
i-INOSITOL	2.0
NICOTINAMIDE	1.0
PYRIDOXINE HCl	1.0
RIBOFLAVIN 5'PHOSPHATE, Na	0.1
THIAMINE HCl	1.0

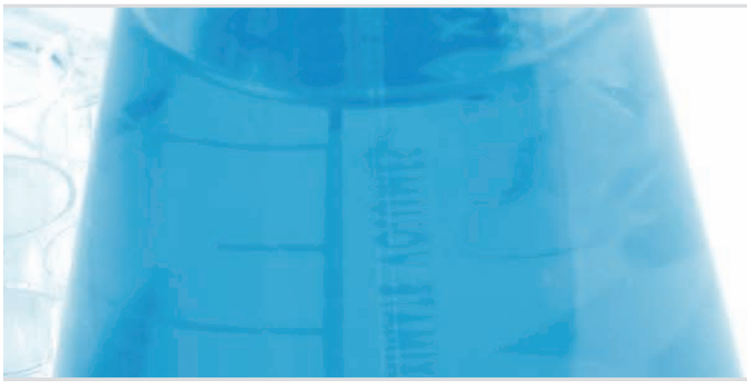
Leibovitz, A., Am. J. Hyg., 78 (1963)

MINIMUM ESSENTIAL MEDIA - EAGLE (MEM)

Product	MEM Earle's Salts Base	MEM Earle's Salts Base With Non-Essential Amino Acids	MEM Hanks' Salts Base	MEM Earle's Salts Base Conc. 10x	MEM For Suspensions
Catalogue No. Component	01-025-1* mg/liter	01-040-1* mg/liter	01-035-1* mg/liter	01-025-5*+ mg/liter	01-045-1* mg/liter
$CaCl_2{\cdot}2H_2O$	264.92	264.92	185.44	2649.2	-
KCl	400.0	400.0	400.0	4000.0	400.0
KH_2PO_4	-	-	60.0	-	-
$MgCl_2{\cdot}6H_2O$	-	-	100.0	-	-
$MgSO_4{\cdot}7H_2O$	200.0	200.0	100.0	2000.0	200.0
NaCl	6800.0	6800.0	8000.0	68000.0	6800.0
$NaHCO_3$	2200.0	2200.0	350.0	-	2200.0
$NaH_2PO_4{\cdot}H_2O$	140.0	140.0	-	1400.0	1400.0
$Na_2HPO_4{\cdot}7H_2O$	-	-	90.0	-	-
GLUCOSE	1000.0	1000.0	1000.0	10000.0	1000.0

Product	MEM Earle's Salts Base	MEM Earle's Salts Base With Non-Essential Amino Acids	MEM Hanks' Salts Base	MEM Earle's Salts Base Conc. 10x	MEM For Suspensions
Catalogue No. Component	01-025-1* mg/liter	01-040-1* mg/liter	01-035-1* mg/liter	01-025-5*+ mg/liter	01-045-1* mg/liter
PHENOL RED	10.0	10.0	10.0	100.0	10.0
L-ALANINE	-	8.9	-	-	-
L-ARGININE HCl	126.0	126.0	126.0	1260.0	126.0
L-ASPARAGINE·H_2O	-	15.0	-	-	-
L-ASPARTIC ACID	-	13.3	-	-	-
L-CYSTINE	24.0	24.0	24.0	240.0	24.0
L-GLUTAMIC ACID	-	14.7	-	-	-
L-GLUTAMINE	292.0*	292.0*	292.0*	-	292.0*
GLYCINE	-	7.5	-	-	-
L-HISTIDINE HCl·H_2O	42.0	42.0	42.0	420.0	42.0
L-ISOLEUCINE	52.0	52.0	52.0	520.0	52.0
L-LEUCINE	52.0	52.0	52.0	520.0	52.0
L-LYSINE HCl	72.5	72.5	72.5	725.0	72.5
L-METHIONINE	15.0	15.0	15.0	150.0	15.0
L-PHENYLALANINE	32.0	32.0	32.0	320.0	32.0
L-PROLINE	-	11.5	-	-	-
L-SERINE	-	10.5	-	-	-
L-THREONINE	48.0	48.0	48.0	480.0	48.0
L-TRYPTOPHAN	10.0	10.0	10.0	100.0	10.0
L-TYROSINE	36.0	36.0	36.0	360.0	36.0
L-VALINE	46.0	46.0	46.0	460.0	46.0
D-CALCIUM PANTOTHENATE	1.0	1.0	1.0	10.0	1.0
CHOLINE CHLORIDE	1.0	1.0	1.0	10.0	1.0
FOLIC ACID	1.0	1.0	1.0	10.0	1.0
i-INOSITOL	2.0	2.0	2.0	20.0	2.0
NICOTINAMIDE	1.0	1.0	1.0	10.0	1.0
PYRIDOXAL HCl	1.0	1.0	1.0	10.0	1.0
RIBOFLAVIN	0.1	0.1	0.1	1.0	0.1
THIAMINE HCl	1.0	1.0	1.0	10.0	1.0

* This preparation is without L-Glutamine.
\+ All media concentrates 10X are prepared without Sodium Bicarbonate.
(1) Eagle, H. Science, 130:432(1959).



Nutrient Mixtures F-10, F-12, and DMEM: F-12

Nutrient Mixture Catalogue No. Component	F-10[1] 01-090-1 mg/liter	F-12[2] 01-095-1 mg/liter	DMEM:F-12 (1:1) 01-170-1* mg/liter
$CaCl_2 \cdot 2H_2O$	44.1	44.0	154.76
$CuSO_4 \cdot 5H_2O$	0.0025	0.0025	0.00125
$FeSO_4 \cdot 7H_2O$	0.834	0.834	0.417
$Fe(NO_3)_3 \cdot 9H_2O$	-	-	0.05
KCl	285.0	223.6	311.8
KH_2PO_4	83.0	-	-
$MgCl_2 \cdot 6H_2O$	-	122.0	61.0
$MgSO_4 \cdot 7H_2O$	152.8	-	100.0
NaCl	7400.0	7599.0	6999.5
$NaHCO_3$	1200.0	1176.0	1200.0
$NaH_2PO_4 \cdot H_2O$	-	-	62.5
$Na2HPO_4$	153.7	142.04	71.02
$ZnSO_4 \cdot 7H_2O$	0.0288	0.863	0.4315
D-GLUCOSE	1100.0	1802.0	3151.0
HEPES	-	-	3575.0
HYPOXANTHINE	4.0	4.1	2.05
LINOLEIC ACID	-	0.084	0.042
LIPOIC ACID	0.2	0.2	0.1
PHENOL RED	1.2	1.2	8.1
PUTRESCINE 2HCl	-	0.161	0.0805
SODIUM PYRUVATE	110.0	110.0	55.0
THYMIDINE	0.7	0.73	0.365
L-ALANINE	9.0	8.9	4.45
L-ARGININE HCl	211.0	211.0	147.5
L-ASPARAGINE·H_2O	15.0	15.0	7.505
L-ASPARTIC ACID	13.3	13.3	6.65
L-CYSTEINE HCl·H_2O	35.12	35.12	17.56
L-CYSTINE	-	-	24.0
L-GLUTAMIC ACID	14.7	14.7	7.35
L-GLUTAMINE	146.0	146.0	365.0
GLYCINE	7.51	7.5	18.75
L-HISTIDINE HCl·H_2O	23.0	20.96	31.48
L-ISOLEUCINE	2.6	3.94	54.47
L-LEUCINE	13.0	13.1	59.05
L-LYSINE HCl	29.0	36.5	91.25
L-METHIONINE	4.48	4.48	17.24
L-PHENYLALANINE	5.0	4.96	35.48
L-PROLINE	11.5	34.5	17.25

Nutrient Mixture Catalogue No. Component	F-10[1] 01-090-1 mg/liter	F-12[2] 01-095-1 mg/liter	DMEM:F-12 (1:1) 01-170-1* mg/liter
L-SERINE	10.5	10.5	26.25
L-THREONINE	3.57	11.9	53.45
L-TRYPTOPHAN	0.6	2.04	9.02
L-TYROSINE	1.8	5.4	38.7
L-VALINE	3.5	11.7	52.85
D-BIOTIN	0.024	0.0073	0.00365
D-CA PANTOTHENATE	0.715	0.48	2.24
CHOLINE CHLORIDE	0.698	13.96	8.98
FOLIC ACID	1.32	1.3	2.65
i-INOSITOL	0.541	18.0	12.6
NIACINAMIDE	0.615	0.037	2.02
PYRIDOXAL HCl	-	-	2.0
PYRIDOXINE HCl	0.206	0.062	0.031
RIBOFLAVIN	0.376	0.038	0.219
THIAMINE HCl	1.00	0.34	2.17
VITAMIN B_{12}	1.36	1.36	0.68

* This preparation is without L-Glutamine.

[1] Ham, R.G., Exp. Cell Res., 29: 515-526, (1963).
[2] Ham, R.G., Proc. Nat. Ac. Sci., 53: 288-293, (1965).

Waymouth's MB 752/1 Medium

Catalogue No. 01-110-1 Component	mg/liter
$CaCl_2 \cdot 2H_2O$	120.00
KCl	150.00
KH_2PO_4	80.00
$MgCl_2 \cdot 6H_2O$	240.00
$MgSO_4 \cdot 7H_2O$	200.00
NaCl	6000.00
$NaHCO_3$	2240.00
Na_2HPO_4	300.00
D-GLUCOSE	5000.00
GLUTATHIONE	15.00
HYPOXANTHINE	25.00
PHENOL RED	10.00
L-ARGININE HCl	75.00
L-ASPARTIC ACID	60.00
L-CYSTEINE HCl·H_2O	100.26
L-CYSTINE	15.00
L-GLUTAMIC ACID	150.00
L-GLUTAMINE	350.00
GLYCINE	50.00
L-HISTIDINE HCl·H_2O	164.10
L-ISOLEUCINE	25.00

Catalogue No. 01-110-1 Component	mg/liter
L-LEUCINE	50.00
L-LYSINE HCl	240.00
L-METHIONINE	50.00
L-PHENYLALANINE	50.00
L-PROLINE	50.00
L-THREONINE	75.00
L-TRYPTOPHAN	40.00
L-TYROSINE	40.00
L-VALINE	65.00
ASCORBIC ACID	17.50
D-BIOTIN	0.02
D-CALCIUM PANTOTHENATE	1.00
CHOLINE CHLORIDE	250.00
FOLIC ACID	0.40
i-INOSITOL	0.90
NICOTINAMIDE	1.00
PYRIDOXINE HCl	1.00
RIBOFLAVINE	1.00
THIAMINE HCl	10.00
VITAMIN B_{12}	0.20

Waymouth, Ch., J. Nat. Canc. Inst., 22:1003, (1959).

Schneider's Drosophila Medium

Catalogue No. 01-150-1 with L-Glutamine Component	mg/liter
$CaCl_2$ (anhyd.)	600.00
KCl	1600.00
KH_2PO_4	450.00
$MgSO_4 \cdot 7H_2O$	3700.00
NaCl	2100.00
$NaHCO_3$	400.00
$Na_2HPO_4 \cdot 7H_2O$	1321.00
Alpha-KETOGLUTARIC ACID	200.00
FUMARIC ACID	100.00
D-GLUCOSE	2000.00
MALIC ACID	100.00

Catalogue No. 01-150-1 with L-Glutamine Component	mg/liter
L-CYSTEINE	60.00
L-CYSTINE	100.00
L-GLUTAMIC ACID	800.00
L-GLUTAMINE	1800.00
GLYCINE	250.00
L-HISTIDINE	400.00
L-ISOLEUCINE	150.00
L-LEUCINE	150.00
L-LYSINE HCl	1650.00
L-METHIONINE	800.00
L-PHENYLALANINE	150.00

Catalogue No. 01-150-1 with L-Glutamine Component	mg/liter
SUCCINIC ACID	100.00
TREHALOSE	2000.00
YEASTOLATE	2000.00
ß-ALANINE	500.00
L-ARGININE	400.00
L-ASPARTIC ACID	400.00

Catalogue No. 01-150-1 with L-Glutamine Component	mg/liter
L-PROLINE	1700.00
L-SERINE	250.00
L-THREONINE	350.00
L-TRYPTOPHAN	100.00
L-TYROSINE	500.00
L-VALINE	300.00

Schneider, I., J. Exp. Zool., 156 (1964).

Grace's Insect Cell Medium

Catalogue No. 01-155-1 with L-Glutamine Component	mg/liter
$CaCl_2 \cdot 2H_2O$	1324.00
KCl	2240.00
$MgCl_2 \cdot 6H_2O$	2280.00
$MgSO_4 \cdot 7H_2O$	2780.00
$NaHCO_3$	350.00
$NaH_2P_04 \cdot H_2O$	1013.00
Alpha-KETOGLUTARIC ACID	370.00
FRUCTOSE	400.00
FUMARIC ACID	55.00
D-GLUCOSE	700.00
MALIC ACID	670.00
SUCCINIC ACID	60.00
SUCROSE	26680.00
ß-ALANINE	200.00
L-ALANINE	225.00
L-ARGININE HCL	700.00
L-ASPARAGINE	350.00
L-ASPARTIC ACID	350.00
L-CYSTINE	22.00
L-GLUTAMIC ACID	600.00
L-GLUTAMINE	600.00
GLYCINE	650.00
L-HISTIDINE	2500.00

Catalogue No. 01-155-1 with L-Glutamine Component	mg/liter
L-ISOLEUCINE	50.00
L-LEUCINE	75.00
L-LYSINE HCl	625.00
L-METHIONINE	50.00
L-PHENYLALANINE	150.00
L-PROLINE	350.00
DL-SERINE	1100.00
L-THREONINE	175.00
L-TRYPTOPHAN	100.00
L-TYROSINE	50.00
L-VALINE	100.00
D-BIOTIN	0.01
D-CALCIUM PANTOTHENATE	0.02
CHOLINE CHLORIDE	0.20
FOLIC ACID	0.02
i-INOSITOL	0.02
NIACIN	0.02
p-AMINOBENZOIC ACID	0.02
PYRIDOXINE HCL	0.02
RIBOFLAVIN	0.02
THIAMINE HCL	0.02

Grace, T.C.C., NATURE, 195:788 (1962).

Balanced Salt Solutions

Product	1. Earle's Balanced Salt Solution		2. Hanks' Balanced Salt Solution		3. Spinner Modified Salt Solution
Concentration Catalogue No. Component	1x 02-010-1 g/liter	10x 02-010-5+ g/liter	1x 02-015-1 g/liter	10x 02-015-5+ g/liter	1x 02-030-1 g/liter
$CaCl_2$	0.2	2.0	0.14	1.4	-
KCl	0.4	4.0	0.4	4.0	0.4
KH_2PO_4	-	-	0.06	0.6	-
$MgCl_2{\cdot}6H_2O$	-	-	0.1	1.0	-
$MgSO_4{\cdot}7H_2O$	0.2	2.0	0.1	1.0	0.2
NaCl	6.8	68.0	8.0	80.0	6.8
$NaHCO_3$	2.2	-	0.35	-	2.2
$Na_2HPO_4{\cdot}7H_2O$	-	-	0.09	0.9	-
$NaH_2PO_4{\cdot}H_2O$	0.14	1.4	-	-	1.4
D-GLUCOSE	1.0	10.0	1.0	10.0	1.0
PHENOL RED	0.01	0.1	0.01	0.1	0.01

Product	4. Dulbecco's Phosphate Buffered Saline			5. Alsever's Solution
Concentration Catalogue No. Component	1x 02-023-1 g/liter	10x 02-023-5 g/liter	1x 02-020-1 g/liter	1x 02-045-1 g/liter
$CaCl_2$	-	-	0.1	-
KCl	0.2	2	0.2	-
KH_2PO_4	0.2	2	0.2	-
$MgCl_2{\cdot}6H_2O$	-	-	0.1	-
NaCl	8.0	80.0	8.0	4.2
$Na_2HPO_4{\cdot}7H_2O$	2.17	21.7	2.17	-
SODIUM CITRATE·$2H_2O$	-	-	-	8.0
D-GLUCOSE	-	-	-	20.5
PHENOL RED	-	-	-	-

\+ This product is prepared without Sodium Bicarbonate.

1. Earle, W.R. et al., Natl. Cancer Inst., 4:167. (1943).
2. Hanks, J.H. and Wallace R.E.,Proc. Soc. Exp. Biol. Med., 71:196, (1949).
3. Eagle, H.,Science 130:432, (1959).
4. Dulbecco, R. and Voght M., J. Exp. Med., 98:167, (1954)
5. Chanock, R.M. and Sabin, A.B., J. Immunol., 70:271 (1953).

Concentrated Component Solutions

Amino Acids and Vitamins

Product	BME Amino Acids	BME Vitamins	MEM Amino Acids	MEM Vitamins	MEM Non-Essential Amino Acids
Concentration Catalogue No. Component	100x 01-315-1 g/liter	100x 01-316-1 g/liter	50x 01-325-1 g/liter	100x 01-326-1 g/liter	100x 01-340-1 g/liter
NaCl	-	8.50	-	8.50	-
L-ALANINE	-	-	-	-	0.89
L-ARGININE HCl	2.10	-	6.32	-	-
L-ASPARAGINE·H_2O	-	-	-	-	1.50
L-ASPARTIC ACID	-	-	-	-	1.33
D-BIOTIN	-	0.10	-	-	-
D-CALCIUM PANTOTHENATE	-	0.10	-	0.10	-
CHOLINE CHLORIDE	-	0.10	-	0.10	-
L-CYSTINE	1.20	-	1.20	-	-
FOLIC ACID	-	0.10	-	0.10	-
L-GLUTAMIC ACID	-	-	-	-	1.47
GLYCINE	-	-	-	-	0.75
L-HISTIDINE HCl·H2O	1.05	-	2.10	-	-
i-INOSITOL	-	0.20	-	0.20	-
L-ISOLEUCINE	2.60	-	2.62	-	-
L-LEUCINE	2.60	-	2.62	-	-
L-LYSINE HCl	3.65	-	3.62	-	-
L-METHIONINE	0.75	-	0.75	-	-
NICOTINAMIDE	-	0.10	-	0.10	-
L-PHENYLALANINE	1.65	-	1.65	-	-
L-PROLINE	-	-	-	-	1.15
PYRIDOXAL HCl	-	0.10	-	0.10	-
RIBOFLAVIN	-	0.01	-	0.01	-
L-SERINE	-	-	-	-	1.05
THIAMINE HCl	-	0.10	-	0.10	-
L-THREONINE	2.40	-	2.38	-	-
L-TRYPTOPHAN	0.40	-	0.51	-	-
L-TYROSINE	1.80	-	1.80	-	-
L-VALINE	2.35	-	2.34	-	-

Antibiotic/Antimycotic Solutions

Catalogue No.	Product	Concentration
03-028-1	AMPHOTERICIN B (FUNGIZONE)[2] SOLUTION Amphotericin B[2] Sodium Deoxycholate	 250 microgram/ml 0.5 mM
03-029-1	AMPHOTERICIN B (FUNGIZONE)[2] SOLUTION Amphotericin B[2] Sodium Deoxycholate	 2,500 microgram/ml 5.0 mM
03-030-1	NYSTATIN[1] SUSPENSION IN DPBS	10,000 u/ml
03-031-1	PENICILLIN-STREPTOMYCIN SOLUTION (P·S) Penicillin G Sodium Salt, Streptomycin, In Saline	10,000 u/ml 10.0 mg/ml
03-031-5	PENICILLIN-STREPTOMYCIN SOLUTION CONCENTRATE 10X Penicillin G, Sodium Salt Streptomycin, In Dpbs	 100,000 u/ml 100.0 mg/ml
03-032-1	PENICILLIN-STREPTOMYCIN-NYSTATIN[1] MIXTURE As Above (P·s) Catalogue No. 03-031-1 With the addition of: Nystatin (Mycostatin)[1]	 1,250 u/ml
03-033-1	PENICILLIN-STREPTOMYCIN-AMPHOTERICIN B[2] SOLUTION As Above (P·s) Catalogue No. 03-031-1 With The Addition of: Amphotericin B (Fungizone)[2]	 25.0 microgram/m
03-034-1	PENICILLIN-STREPTOMYCIN-NEOMYCIN SOLUTION As Above (P·s) Catalogue No. 03-031-1 With The Addition of: Neomycin	 10.0 mg/ml
03-035-1	GENTAMYCIN SULPHATE SOLUTION	50.0 mg/ml (as base)
03-049-1	KANAMYCIN SULPHATE SOLUTION	10.0 mg/ml (as base)

(1) Nystatin Is The Generic Name for Mycostatin® Which Is The Registered Trade Name Of E.R. Squibb & Sons.
(2) Amphotericin B Is The Generic Name for Fungizone® Which Is The Registered Trade Name Of E.R. Squibb & Sons.

Powdered Cell Culture Media Formulations

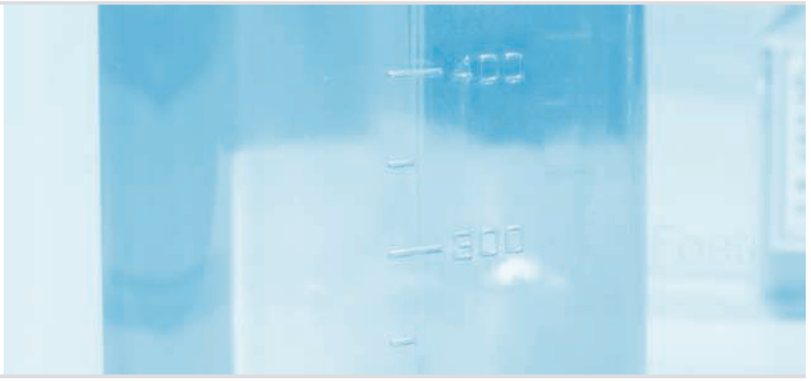
Product Catalogue No. Component	MEM-EARLE'S 11-025-1 mg/liter	MEM-ALPHA 11-042-1 mg/liter	DMEM LOW GLUCOSE 11-050-1 mg/liter	DMEM HIGH GLUCOSE 11-055-1 mg/liter
Calcium Chloride Dihydrate	264.90	264.90	264.90	264.90
Ferric Nitrate Nonahydrate	-	-	0.10	0.10
Potassium Chloride	400.00	400.00	400.00	400.00
Magnesium Sulfate	97.66	97.66	97.66	97.66
Sodium Chloride	6800.00	6800.00	6400.00	6400.00
Sodium Dihydrogen Phosphate	121.74	121.74	108.69	108.69
L-Alanine	-	25.00	-	-
L-Arginine Hydrochloride	126.98	126.98	84.00	84.00
L-Asparagine Monohydrate	-	50.00	-	-
L-Aspartic Acid	-	30.00	-	-
L-Cysteine Hydrochloride Monohydrate	-	100.00	-	-
L-Cystine Dihydrochloride	31.29	31.29	62.58	62.58
L-Glutamic Acid	-	75.00	-	-
L-Glutamine	292.00	292.00	584.00	584.00
Glycine	-	50.00	30.00	30.00
L-Histidine Hydrochloride Monohydrate	42.00	42.00	42.00	42.00
L-Isoleucine	52.00	52.50	104.80	104.80
L-Leucine	52.00	52.50	104.80	104.80
L-Lysine Hydrochloride	72.46	72.46	146.20	146.20
L-Methionine	15.00	15.00	30.00	30.00
L-Phenylalanine	32.00	32.00	66.00	66.00
L-Proline	-	40.00	-	-
L-Serine	-	25.00	42.00	42.00
L-Threonin	48.00	48.00	95.20	95.20
L-Tryptophan	10.00	10.00	16.00	16.00
L-Tyrosine Disodium	51.90	51.90	103.79	103.79
L-Valine	46.00	46.00	93.60	93.60
Ascorbic Acid	-	50.00	-	-
D-Biotin	-	0.10	-	-
Choline Chloride	1.00	1.00	4.00	4.00
Folic Acid	1.00	1.00	4.00	4.00
i-Inositol	2.00	2.00	7.00	7.00
Nicotinamide	1.00	1.00	4.00	4.00
D-Calcium Pantothenate	1.00	1.00	4.00	4.00
Pyridoxal Hydrochloride	1.00	1.00	4.00	4.00

Product	MEM-EARLE'S	MEM-ALPHA	DMEM LOW GLUCOSE	DMEM HIGH GLUCOSE
Catalogue No. / Component	11-025-1 mg/liter	11-042-1 mg/liter	11-050-1 mg/liter	11-055-1 mg/liter
Riboflavin	0.10	0.10	0.40	0.40
Thiamine Hydrochloride	1.00	1.00	4.00	4.00
Vitamin B-12	-	1.36	-	-
D-Glucose	1000.00	1000.00	1000.00	4500.00
Lipoic Acid	-	0.20	-	-
Phenol Red Sodium	10.20	10.20	15.34	15.34
Sodium Pyruvate	-	110.00	110.0	-

Powdered Cell Culture Media Formulations

Product	M-199 EARLE'S	NUT. F-10	NUT. F-12	DMEM F-12 (1:1)
Catalogue No. / Component	11-080-1 mg/liter	11-090-1 mg/liter	11-095-1 mg/liter	11-170-1 mg/liter
Calcium Chloride Dihydrate	264.90	44.10	44.10	154.52
Cupric Sulfate Pentahydrate	-	0.0025	0.002	0.00125
Ferric Nitrate Nonahydrate	0.72	-	-	0.05
Ferrous Sulfate Heptahydrate	-	0.834	0.834	0.417
Potassium Chloride	400.00	285.00	223.65	311.83
Potassium Dihydrogen Phosphate	-	83.00	-	-
Magnesium Chloride	-	-	57.20	28.60
Magnesium Sulfate	97.66	74.62	-	48.83
Sodium Bicarbonate	-	-	-	-
Sodium Chloride	6800.00	7400.00	7599.00	6999.50
Sodium Dihydrogen Phosphate	121.74	-	-	54.35
Disodium Hydrogen Phosphate	-	153.73	142.12	71.06
Zinc Sulfate Heptahydrate	-	0.0288	0.863	0.432
L-Alanine	25.00	8.91	8.91	4.45
L-Arginine Hydrochloride	70.00	211.00	210.7	147.35
L-Asparagine	-	-	15.01	-
L-Asparagine Monohydrate	-	15.00	-	7.51
L-Aspartic Acid	30.00	13.30	13.30	6.66
L-Cysteine Hydrochloride Monohydrate	0.11	39.10	35.12	17.56
L-Cystine Dihydrochloride	26.08	-	-	31.29
L-Glutamic Acid	75.00	14.70	14.71	7.36
L-Glutamine	100.00	146.20	146.20	365.10
Glycine	50.00	7.51	7.51	18.76

Product	M-199 EARLE'S	NUT. F-10	NUT. F-12	DMEM F-12 (1:1)
Catalogue No. / Component	11-080-1 mg/liter	11-090-1 mg/liter	11-095-1 mg/liter	11-170-1 mg/liter
L-Histidine Hydrochloride Monohydrate	21.88	22.98	20.96	31.48
Hydroxy-L-Proline	10.00	-	-	-
L-Isoleucine	20.00	2.60	3.936	54.37
L-Leucine	60.00	13.10	13.10	58.96
L-Lysine Hydrochloride	70.00	29.30	36.54	91.37
L-Methionine	15.00	4.48	4.48	17.24
L-Phenylalanine	25.00	4.96	4.96	35.48
L-Proline	40.00	11.50	34.53	17.27
L-Serine	25.00	10.50	10.50	26.26
L-Threonine	30.00	3.57	11.91	53.56
L-Tryptophan	10.00	0.60	2.042	9.02
L-Tyrosine Disodium	57.66	2.61	7.836	55.81
L-Valine	25.00	3.50	11.71	52.66
Ascorbic Acid	0.05	-	-	-
Biotin	0.01	0.024	0.007	0.00367
Choline Chloride	0.50	0.697	13.96	8.98
Ergocalciferol	0.10	-	-	-
Folic Acid	0.01	1.32	1.32	2.662
i-Inositol	0.05	0.541	18.02	12.51
Menadione	0.01	-	-	-
Nicotinamide	0.025	0.615	0.037	2.018
Nicotinic Acid	0.025	-	-	-
D-Calcium Pantothenate	0.01	0.715	0.238	2.12
p-Aminobenzoic Acid	0.05	-	-	-
Pyridoxal Hydrochloride	0.025	-	-	2.00
Pyridoxine Hydrochloride	0.025	0.206	0.062	0.031
Riboflavin	0.01	0.376	0.038	0.22
Thiamine Hydrochloride	0.01	1.012	0.337	2.17
Vitamin A Acetate	0.14	-	-	-
Vitamin B-12	-	1.36	1.36	0.679
Vitamin E Phosphate Disodium	0.01	-	-	-
Adenine Sulfate	10.00	-	-	-
Adenosine Triphosphate Sodium	1.00	-	-	-
Adenylic Acid	0.20	-	-	-
Cholesterol	0.20	-	-	-
Deoxyribose	0.50	-	-	-



Product	M-199 EARLE'S	NUT. F-10	NUT. F-12	DMEM F-12 (1:1)
Catalogue No. Component	11-080-1 mg/liter	11-090-1 mg/liter	11-095-1 mg/liter	11-170-1 mg/liter
D-Glucose	1000.00	1100.00	1801.60	3150.80
Glutathione (Reduced)	0.05	-	-	-
Guanine	0.30	-	-	-
Hypoxanthine Sodium	0.399	5.43	5.43	2.715
Linoleic Acid	-	-	0.084	0.0421
Lipoic Acid	-	0.20	0.206	0.103
Phenol Red (Sodium)	20.40	1.23	1.27	8.305
Putrescine Dihydrochloride	-	-	0.161	0.0806
D-Ribose	0.50	-	-	-
Sodium Acetate	50.00	-	-	-
Sodium Pyruvate	-	110.00	110.10	55.05
Thymidine	-	0.727	0.727	0.360
Thymine	0.30	-	-	-
Tween 80	20.00	-	-	-
Uracil	0.30	-	-	-
Xanthine Sodium	0.344	-	-	-
Hepes	-	-	-	15mM

Powdered Cell Culture Media Formulations

Product	McCOY'S 5A	ISCOVE'S	MEM WITH NEAA	RPMI 1640
Catalogue No. Component	11-075-1 mg/liter	11-058-1 mg/liter	11-040-1 mg/liter	11-100-1 mg/liter
Calcium Chloride Dihydrate	132.46	218.56	264.86	-
Calcium Nitrate Tetrahydrate	-	-	-	100.00
Potassium Chloride	400.00	330.00	400.00	400.00
Potassium Nitrate	-	0.076	-	-
Potassium Dihydrogen Phosphate	-	-	-	-
Magnesium Chloride	-	-	-	-
Magnesium Sulfate	97.66	97.66	97.66	48.83
Na2 SeO3.5H20	-	0.0173	-	-
Sodium Chloride	6460.00	4505.00	6800.00	6000.00
Sodium Dihydrogen Phosphate	504.35	108.69	121.73	-
Disodium Hydrogen Phosphate	-	-	-	800.49
DL-Alanine	-	-	-	-
L-Alanine	13.90	25.00	8.90	-

Product	McCOY'S 5A	ISCOVE'S	MEM WITH NEAA	RPMI 1640
Catalogue No. Component	11-075-1 mg/liter	11-058-1 mg/liter	11-040-1 mg/liter	11-100-1 mg/liter
L-Arginine	-	-	-	-
L-Arginine Hydrochloride	42.10	84.00	126.40	241.86
L-Asparagine	45.00	-	-	-
L-Asparagine Monohydrate	-	28.40	15.00	50.00
L-Aspartic Acid	19.97	30.00	13.30	20.00
L-Cysteine	31.50	-	-	-
L-Cysteine Hydrochloride Monohydrate	-	-	-	-
L-Cystine Dihydrochloride	-	91.24	28.39	65.19
L-Glutamic Acid	22.10	75.00	14.70	20.00
L-Glutamine	219.20	584.00	292.00	300.00
Glycine	7.50	30.00	7.50	10.00
L-Histidine Hydrochloride Monohydrate	20.96	42.00	41.93	20.27
Hydroxy-L-Proline	19.70	-	-	20.00
L-Isoleucine	39.36	104.80	52.00	50.00
L-Leucine	39.36	104.80	52.00	50.00
L-Lysine	-	-	-	-
L-Lysine Hydrochloride	36.50	146.20	73.06	40.00
DL-Methionine	-	-	-	-
L-Methionine	14.90	30.00	15.00	15.00
DL-Phenylalanine	-	-	-	-
L-Phenylalanine	16.50	66.00	33.00	15.00
L-Proline	17.30	40.00	11.50	20.00
L-Serine	26.30	42.00	10.50	30.00
DL-Threonine	-	-	-	-
L-Threonine	17.90	95.20	48.00	20.00
DL-Tryptophan	-	-	-	-
L-Tryptophan	3.10	16.00	10.00	5.00
L-Tyrosine	-	-	-	-
L-Tyrosine Disodium	26.10	103.79	44.74	28.83
DL-Valine	-	-	-	-
L-Valine	17.60	93.60	46.86	20.00
Ascorbic Acid	0.50	-	-	-
D-Biotin	0.20	0.013	-	0.20
Choline Chloride	5.00	4.00	1.00	3.00
Folic Acid	10.00	4.0	1.00	1.00
i-Inositol	36.00	7.00	2.00	35.00
Nicotinamide	0.50	4.00	1.00	1.00
Nicotinic Acid	0.50	-	-	-

Product	McCOY'S 5A	ISCOVE'S	MEM WITH NEAA	RPMI 1640
Catalogue No. Component	11-075-1 mg/liter	11-058-1 mg/liter	11-040-1 mg/liter	11-100-1 mg/liter
D-Calcium Pantothenate	0.20	4.00	1.00	0.25
p-Aminobenzoic Acid	1.00	-	-	1.00
Pyridoxal Hydrochloride	0.50	4.00	1.00	-
Pyridoxine Hydrochloride	-	-	-	1.00
Riboflavin	0.20	0.40	0.10	0.20
Riboflavin 5'-Phosphate	-	-	-	-
Thiamine Hydrochloride	0.20	4.00	1.00	1.00
Thiamine Monophosphate Dihydrate	-	-	-	-
Vitamin B-12	2.00	0.013	-	0.005
Bactopeptone	600.00	-	-	-
D(+) Galactose	-	-	-	-
D-Glucose	3000.00	4500.00	1000.00	2000.00
Glutathione (Reduced)	0.50	-	-	1.00
Hypoxanthine Sodium	-	-	-	-
Phenol Red (Sodium)	10.20	15.34	10.00	5.1
Sodium Pyruvate	-	110.00	-	-
HEPES	-	25 mM	-	-

Crystalline Trypsin Solution & Soybean Trypsin Inhibitor Solution

Product	CRYSTALLINE TRYPSIN Solution	SOYBEAN TRYPSIN INHIBITOR
Catalogue No. Component	03-047-1 gm/l	03-048-1 gm/l
CRYSTALLINE TRYPSIN	0.2	-
SOYBEAN TRYPSIN INHIBITOR	-	5.0
POLYVINYL PYRROLIDONE	5.0	-
TRICINE	3.58	-
NaCl	8.0	8.0
KCl	0.4	0.2
Na2HPO4 7H2O	-	2.16
KH2PO4	-	0.2
PHENOL RED	-	-

TRYPSIN SOLUTIONS

Product	TRYPSIN Solution "A"	TRYPSIN Solution "B"	TRYPSIN EDTA Solution "A"	TRYPSIN EDTA Solution "B"	TRYPSIN EDTA Solution "C"	TRYPSIN Solution "B" Conc. 10x	TRYPSIN EDTA Conc. 10x
Catalogue No. Component	03-045-1 gm/l	03-046-1 gm/l	03-050-1 gm/l	03-052-1 gm/l	03-053-1 gm/l	03-046-5 gm/l	03-051-5 gm/l
TRYPSIN 1:250	2.5	2.5	2.5	2.5	0.5	25.0	5.0
EDTA Na2 2H2O	-	-	0.2	0.5	0.2	-	2.0
NaCl	8.0	8.0	8.0	8.0	8.0	80.0	8.5
KCl	0.2	0.2	0.4	0.4	0.2	2.0	-
Na2HPO4	1.15	1.15	-	-	1.15	11.5	-
KH2PO4	0.2	0.2	-	-	0.2	2.0	-
D-GLUCOSE	-	-	1.0	1.0	-	-	-
PHENOL RED	-	-	0.01	0.01	0.01	-	-
NaHCO3	-	-	0.35	0.35	-	-	-
CaCl2·2H2O	0.13	-	-	-	-	-	-
MgCl2·6H2O	0.1	-	-	-	-	-	-

CERTIFICATES

IQNet®

THE INTERNATIONAL CERTIFICATION NETWORK

CERTIFICATE

IQNet and
THE STANDARDS INSTITUTION OF ISRAEL
hereby certify that the organization

Biological Industries Israel Beit Haemek Ltd.
KIBBUTZ BEIT HAEMEK
for the following field of activities
MANUFACTURE OF PRODUCTS FOR ANIMAL CELL CULTURE
AND MOLECULAR BIOLOGY.
LABORATORY TESTING SERVICES.
(SEE APPENDIX)

has implemented and maintains a

Quality Management System

which fulfills the requirements of the following standard/s

ISO 9001:2000

Issued on : 15 . 03 . 2007
Date of expiration: 31 . 05 . 2010
Date of initial approval: 01 . 02 . 1995

Registration number: IL- 27738

René Wasmer
President of IQNet

Doron Tamir
Director General, SII

IQNet (12/07)

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IQNet®

THE INTERNATIONAL CERTIFICATION NETWORK

CERTIFICATE

IQNet and
THE STANDARDS INSTITUTION OF ISRAEL
hereby certify that the organization

Biological Industries Israel Beit Haemek Ltd.
KIBBUTZ BEIT HAEMEK
for the following field of activities
MANUFACTURE OF PRODUCTS FOR ANIMAL CELL CULTURE
AND MOLECULAR BIOLOGY.
LABORATORY TESTING SERVICES.
(SEE APPENDIX)

has implemented and maintains a

Quality Management System

which fulfills the requirements of the following standard/s

ISO 13485:2003

Issued on : 01 . 04 . 2007
Date of expiration: 30 . 04 . 2010
Date of initial approval: 10 . 04 . 2006

Registration number: IL- 40846

René Wasmer
President of IQNet

Doron Tamir
Director General, SII

IQNet (12/07)

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IQNet is represented in the USA by: AFAQ AFNOR, CISQ, DQS, NSAI Inc., QMI and SAI Global.
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CERTIFIED QMS
SI ISO 9001: 2000
THE STANDARDS INSTITUTION OF ISRAEL

Certificate

This is to certify that the
Quality Management System
of

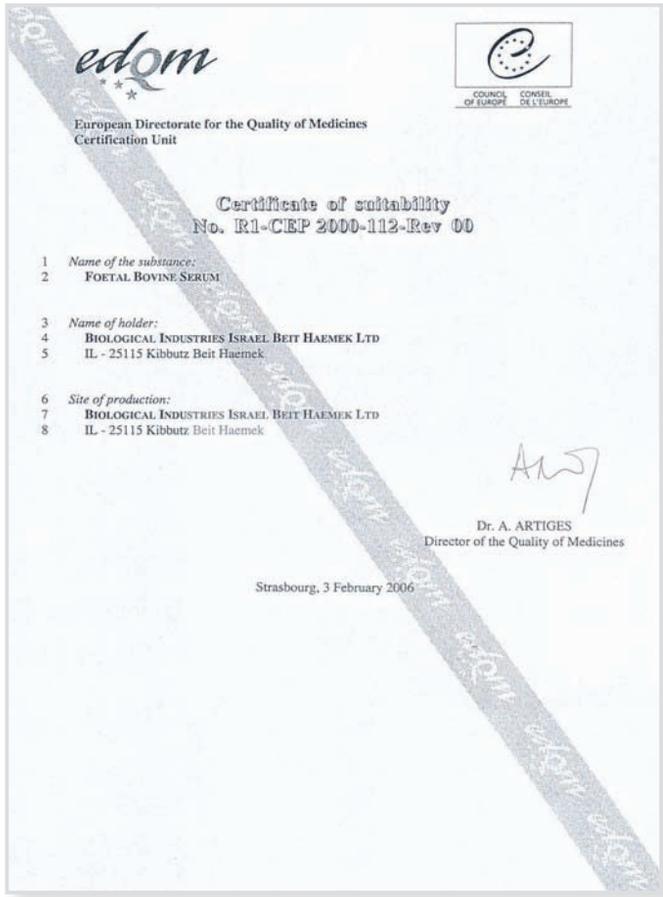
Biological Industries Israel Beit Haemek Ltd.
KIBBUTZ BEIT HAEMEK
has been audited by SII and found to comply with the Quality Management Standard SI ISO 9001: 2000
scope:

MANUFACTURE OF PRODUCTS FOR ANIMAL CELL CULTURE
AND MOLECULAR BIOLOGY
LABORATORY TESTING SERVICES
(SEE APPENDIX)

The Certificate is granted in accordance with SII's Rules for the Certification of Quality Systems (SII procedure-QP). The validity of the Certificate is subject to the continuous maintenance of the Quality System according to the above standard, and the follow-up surveillance performed by SII. Further clarifications regarding the scope of the certificate and applicability of ISO 9001:2000 requirements may be obtained by consulting the organization.

Date of initial approval: 01 02 1995 — *License No.* 27738
Date of expiration 31 05 2010 — *Date of issue:* 15 03 2007

THE STANDARDS INSTITUTION OF ISRAEL — מתי — Dr. Dror Tamir, Director General — IQNet — Accredited by the RvA



edqm

COUNCIL OF EUROPE — CONSEIL DE L'EUROPE

European Directorate for the Quality of Medicines
Certification Unit

Certificate of suitability
No. R1-CEP 2000-112-Rev 00

1 *Name of the substance:*
2 FOETAL BOVINE SERUM

3 *Name of holder:*
4 BIOLOGICAL INDUSTRIES ISRAEL BEIT HAEMEK LTD
5 IL - 25115 Kibbutz Beit Haemek

6 *Site of production:*
7 BIOLOGICAL INDUSTRIES ISRAEL BEIT HAEMEK LTD
8 IL - 25115 Kibbutz Beit Haemek

Dr. A. ARTIGES
Director of the Quality of Medicines

Strasbourg, 3 February 2006

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REPRESENTATION OF COMPANIES IN THE DOMESTIC MARKET

Companies Represented by Biological Industries in the Domestic Market

To fully exploit our established expertise in the life science area, we maintain close relationships with the Israeli scientific community. Biological Industries is a leading cell culture innovation provider for Israeli customers. We represent several leading life science research, diagnostic and bioprocess innovation providers to domestic clients. Israel is one of the largest research markets per capita, based on a high level of life science scientific research.

Promega (www.promega.com)
Molecular Biology Leadership
Leading products for life science research, particularly in genomics, proteomics, and cellular analysis. Promega's products are also used to support molecular diagnostics and human identification.
Promega's products for advanced molecular biology research are a part of Biological Industries' integrative approach of providing a full service package to academia and biopharma customers in the local market. We have a long term mutually beneficial relationship with Promega with the constant provision of innovation and interactive technical support at the highest level.
Promega's clinical and academic labs loyal customers in Israel serve as beta sites for new developments and product introductions. Several research collaborations were catalyzed by BI between Israeli scientists and Promega.
New: MaxWell- a user friendly microrobotic system from Promega has been well recognized as a solution for high throughput DNA testing.

Sartorius-Stedim (www. sartorius-stedim.com)
Global Leader in Bioprocess and Filtration Applications
New: Single Use Cell Culture Bioreactors for laboratory applications and scale up. Sartorius' recent entry into the disposable bioreactors market, with the CultiFlask (350, 1000 ml), CeLLine for labscale (20-50 ml) and Biostat® CultiBag RM (0.5–and up to 300 L), relies on rocking motion mixing technology of Wave Biotech AG and integrates with Sartorius BBI controllers.

Ansell (www.ansellpro.com)
Global leader in barrier protective solutions, specialist in surgical, examination, industrial and household gloves and protective clothing.

Applichem AG (www.applichem.de)
Provides high quality cost effective biochemicals and reagents for research.

Biovison Inc. (www.biovision.com)
Provides cell biology products. Offers a wide variety of products including reagents, bioassay kits, antibodies, recombinant proteins, and other research tools for studying apoptosis, cell signaling pathways, cell proliferation, metabolism, cellular stress, cell damage and repair, cholesterol and obesity, Protein A/G/L, multi-color fluorescence proteins and gene regulation, etc.

CellNTech (www.cellntec.com)
Specializes in the use of novel Progenitor Cell Targeting (PCT) technology to provide a wide range of epithelial in vitro models and custom dermatological testing services.

Devyser AB (www.devyser.com)
Specializes in diagnostic kits and reagents based on DNA analytical procedures, including PCR. The main areas of application for these products are prenatal diagnostics and clinical genetics.
We are constantly looking for more innovative molecular diagnostic suppliers to represent in the domestic market.

Jena Bioscience (www.jenabioscience.com)
Provides innovative reagents and technologies for the life science market.

Kreatech (www.kreatech.com)
A molecular diagnostics company focused on innovative detection products. These are used for diagnostic and research applications in the life sciences and healthcare industry. These applications include cytogenetics, microarrays, and proteomics.
Has particular expertise in the areas of oncology, haematology and pre-natal diagnostics.
Has a well established range of FISH probes and offers a new range of repeat-free probes for superior results in cytogenetic analysis.

Mobitec (www.mobitec.de)

Promocell AG (www.promocell.com)
Supports the trend towards carrying out in-vitro cell culture tests using normal human primary cells.
Provides proprietary cell lines and special reagents for vascular diseases and primary and adult stem cells research.
Provides important cell types, together with serum-free or low-serum cell culture media.

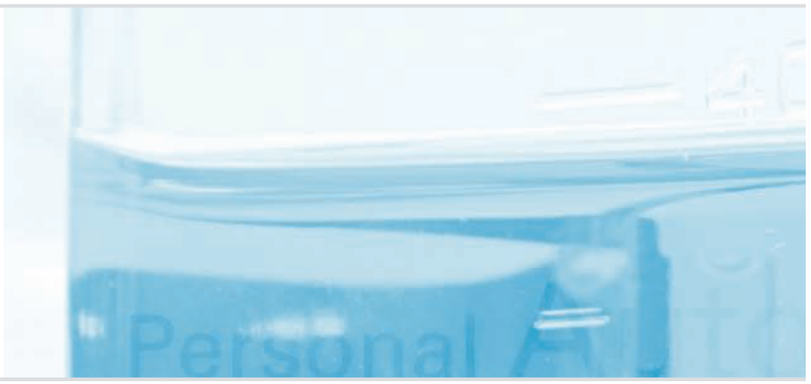
Ray Biotech (www.raybiotech.com)
Provides high quality antibody and protein array technology to efficiently analyze the concurrent expression and function of hundreds of proteins, involved in inflammation, angiogenesis, apoptosis, cell growth, and signal transduction from a single biological sample.

Shun tay (www.shuntaihoptai.com)
Specializes in manufacturing all types of working gloves.

Sterilator

Zen-Bio Inc.- (www.zen-bio.com)
Provides products for disease models and cosmetics.
Provider of human cell lines, reagents and research tools for the study of human metabolic disease. Offers a variety of assays to test compounds for potential as diabetes and obesity therapies.

ALPHABETICAL INDEX BY PRODUCT NAME



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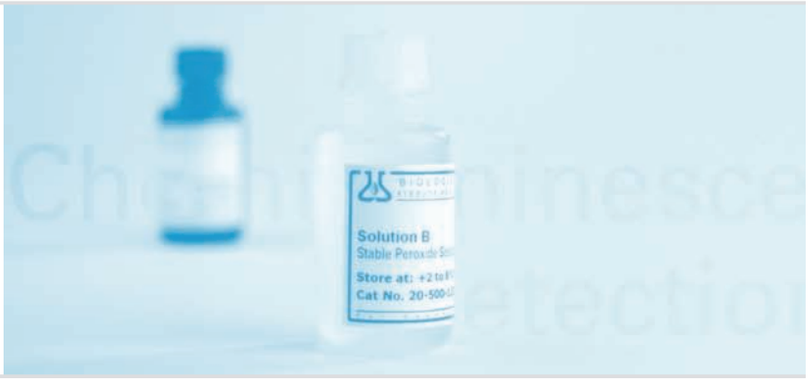
Dulbecco's Modified Eagle Medium (DMEM), without D-Glucose, without Sodium Pyruvate, without L-Glutamine	01-057-1	19



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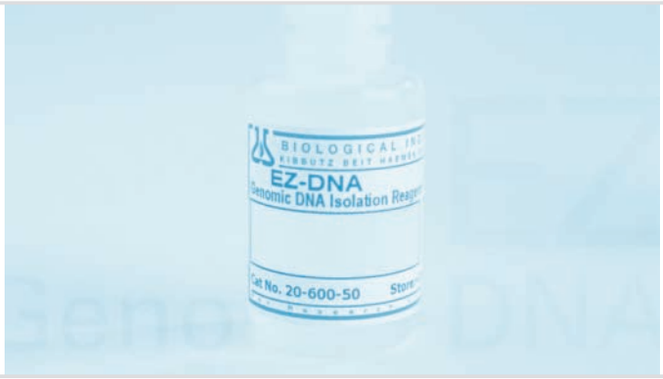

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01-939-1	Quick Stain	90
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03-032-1	Penicillin-Streptomycin Nystatin Solution, 10,000 units/ml Penicillin G Sodium Salt, 10mg/ml Streptomycin Sulfate, 1,250 units/ml Nystatin	72-73, 136
03-033-1	Penicillin-Streptomycin Amphotericin B Solution, 10,000 units/ml Penicillin G Sodium Salt, 10mg/ml Streptomycin Sulfate, 25 microgram/ml Amphotericin B	72-73, 136
03-034-1	Penicillin-Streptomycin Neomycin Solution, 10,000 units/ml Penicillin G Sodium Salt, 10mg/ml Streptomycin Sulfate, 10mg/ml Neomycin Sulfate	72-73, 136
03-035-1	Gentamycin Sulfate Solution, 50 mg/ml	73
03-036-1	BIOMYC-1 Antibiotic Solution 100X Conc.	96
03-037-1	BIOMYC-2 Antibiotic Solution 100X Conc.	96
03-038-1	BIOMYC-3 Antibiotic Solution 100X Conc.	97
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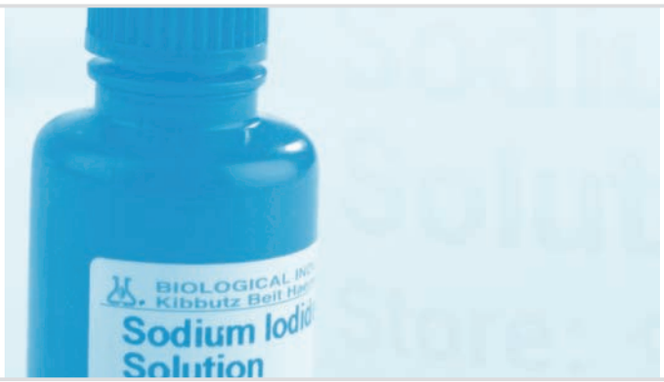

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