



## Toolbox reagents



Anti-tag conjugates  
Anti-species  
Affinity reagents

# HTRF<sup>®</sup> reagent toolbox

HTRF<sup>®</sup> reagent toolbox contains a range of useful tools for increasing assay design flexibility, particularly if protein-specific reagents are not available.

## Description

Tagged biomolecules are a key element in studying molecular interactions and offer researchers flexibility in assay design. The most frequently used tags consist of proteinic or peptidic structures such as GST, 6HIS, c-myc, FLAG<sup>®</sup>, and HA. Small organic motifs like biotin and dinitrophenyl (DNP) are also widely used for developing assays. The HTRF<sup>®</sup> reagent toolbox offers a comprehensive selection of conjugated binders – anti-tag antibodies, streptavidins, lectins – for detecting this broad diversity of motifs. The toolbox also includes secondary murine, sheep, rabbit, and human antibodies, as well as immunoglobulin binding proteins. Streptavidin is available conjugated to Eu<sup>3+</sup> cryptate and to XL665. The SA-XL<sup>entl</sup> reagent represents a brighter XL665-conjugated streptavidin for assays requiring high sensitivity.

## Anti-tag and secondary antibody characteristics:

Motif/Species	Antibody	Species and Subtype	Specificity
GST	MAb GSS11	Mouse IgG2a	Schistosoma japonicum GST
6HIS	MAb HIS-1	Mouse IgG2a	HexaHistidine peptide
c-myc	MAB 9E10	Mouse IgG1	EQKLISEEDL peptide
FLAG <sup>®</sup>	MAB M2	Mouse IgG1	DYKDDDDK peptide
HA	MAB HAS01	Mouse IgG1	YPYDVPDYA peptide
DNP	MAB 265.5	Mouse IgG1	2,4-dinitrophenyl motif
Mouse immunoglobulins	PAb	Rabbit	Mouse Igs
Rabbit immunoglobulins	PAb	Goat	Rabbit Igs
Human immunoglobulins	PAb	Goat	Human Fc Igs

## Features

- High affinity monoclonal and polyclonal antibodies
- High-grade streptavidins (see page 56)
- Resistant to most buffer conditions and additives (e.g. DMSO, pH, chelators, ionic strength)
- Compatible with membrane and cell-based assays
- Lyophilized packaging for easy handling and long term storage
- Proven batch-to-batch reproducibility
- High quality custom labeling and assay development services available

## THE ADVANTAGES

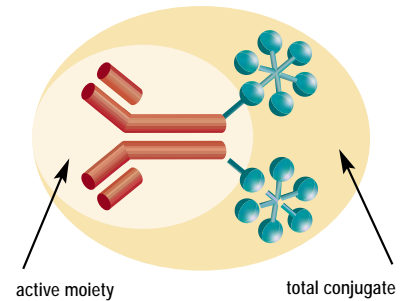
- A unique set of reagents for developing homogeneous high throughput assays.
- Allows flexible and straightforward scaling up for primary and secondary screening phases.
- Enables multiple target developments and assay configurations, such as protein:protein and nuclear receptor screening, receptor dimerization, protease and investigation of other enzymatic processes.

## HTRF<sup>®</sup> reagent toolbox

### Principle of use and definitions

All reagents in the HTRF<sup>®</sup> toolbox are supplied with reference to a 384 low volume well format, for a final assay volume of 20  $\mu$ L. HTRF<sup>®</sup> is particularly well suited to miniaturization, and the number of wells specified for each product reference can be higher depending on the level of miniaturization.

Reference tables also give the average quantities of active moiety per vial. Active moiety is defined as the active part of a conjugate (e.g. antibody, streptavidin), as shown beside:

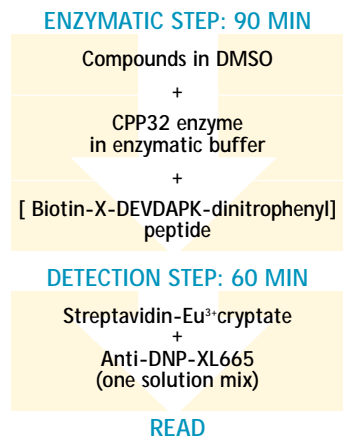


For instance, antibodies conjugated to XL665 are supplied on the basis of 20 ng of antibody per well. However, their equivalence in terms of average conjugate quantity per well depends on the molar ratio antibody/XL665, and therefore varies from one toolbox reagent to another. In practice, the active moiety amount is generally preferred to that of total conjugate as a basis for calculating assay development. This is due to the fact that the label moiety does not influence the interaction studied *per se*. The average conjugate quantity per well is information that reflects overall biological material content. For Eu<sup>3+</sup> cryptate, biotin, and DNP conjugates, the total conjugate amount equals that of the active moiety, since the molecular weight of the label is negligible.

### Recommended quantities of Eu<sup>3+</sup> cryptate and XL665 conjugate:

Most assays can be run within the nanomolar range. However, as a tracer, Eu<sup>3+</sup> cryptate conjugates must not be excessive in order to prevent reader saturation and an unacceptable level of background. In most cases, an Eu<sup>3+</sup> cryptate concentration of 4 to 5 nM is appropriate, and will typically generate 620 nm fluorescence of about 40,000 cps on RUBYstar, the HTRF<sup>®</sup> reference reader. As an example, for an antibody conjugated to Eu<sup>3+</sup> cryptate with a molar ratio of 5 cryptates/Ab, the recommended value would be close to 1 nM of antibody. The XL665 conjugate must match its assay counterpart as closely as possible so that the maximum number of biomolecules can be tagged with the XL665 acceptor. Thus, to detect a GST-tagged molecule at an assay concentration of 20 nM, the concentration of anti-GST-XL665 should be equimolar or higher. The actual amount will depend on the assay configuration and the degree of miniaturization.

Reagents from the HTRF<sup>®</sup> toolbox can be used in multiple configurations. A number of assays can be developed using a simple one-step homogeneous protocol in which interaction occurs simultaneously with detection. The example given here – a study published by Pr eaudat et al. - describes a two-step protocol for quantifying caspase 3 substrate cleavage (DEVD). Like most HTRF<sup>®</sup> protease systems, the assay is set up using a universal cassette detection system made of streptavidin-cryptate and anti-DNP-XL665. It is entirely homogeneous and runs in a single plate.



## HTRF® reagent toolbox

### Eu<sup>3+</sup> cryptate conjugates

All Eu<sup>3+</sup> cryptate conjugates are calibrated on RUBYstar, the HTRF® reference reader, in order to generate fluorescence of 40,000 cps on the 620 nm channel on average.

Anti-tab toolbox	Size	Active moiety per vial (average)	Cat#
Anti-GST K	5,000 tests	10 µg	61GSTKLA
Anti-GST K	20,000 tests	40 µg	61GSTKLB
Anti-6HIS K	5,000 tests	10 µg	61HISKLA
Anti-6HIS K	20,000 tests	40 µg	61HISKLB
Anti-c-myc K	5,000 tests	15 µg	61MYCKLA
Anti-c-myc K	20,000 tests	60 µg	61MYCKLB
Anti-FLAG® K	5,000 tests	5 µg	61FG2KLA
Anti-FLAG® K	20,000 tests	20 µg	61FG2KLB
Anti-HA K	5,000 tests	15 µg	610HAKLA
Anti-HA K	20,000 tests	60 µg	610HAKLB
Anti-DNP K	5,000 tests	10 µg	61DNPKLA
Anti-DNP K	20,000 tests	40 µg	61DNPKLB

Anti-immunoglobulins	Size	Active moiety per vial (average)	Cat#
Anti-mouse IgG-K	5,000 tests	7.5 µg	61PAMKLA
Anti-mouse IgG-K	20,000 tests	30 µg	61PAMKLB
Anti-rabbit IgG-K	5,000 tests	7.5 µg	61PARKLA
Anti-rabbit IgG-K	20,000 tests	30 µg	61PARKLB
Anti-human IgG-K	5,000 tests	10 µg	61HFCKLA
Anti-human IgG-K	20,000 tests	40 µg	61HFCKLB

Affinity sytem reagents	Size	Active moiety per vial (average)	Cat#
Streptavidin K	5,000 tests	40 µg	610SAKLA
Streptavidin K	20,000 tests	160 µg	610SAKLB
Biotin K	5,000 tests	0.5 µg	61BTNKLA
Biotin K	20,000 tests	2 µg	61BTNKLB
Protein A K	5,000 tests	3.5 µg	61PRAKLA*
Protein A K	20,000 tests	14 µg	61PRAKLB*

### XL665 conjugates

Anti-tab toolbox	Size	Active moiety per vial (average)	Cat#
Anti-GST XL665	5,000 tests	100 µg	61GSTXLA
Anti-GST XL665	20,000 tests	400 µg	61GSTXLB
Anti-6HIS XL665	5,000 tests	100 µg	61HISXLA
Anti-6HIS XL665	20,000 tests	400 µg	61HISXLB
Anti-c-myc XL665	5,000 tests	100 µg	61MYCXLA
Anti-c-myc XL665	20,000 tests	400 µg	61MYCXLB
Anti-FLAG® XL665	5,000 tests	100 µg	61FG2XLA
Anti-FLAG® XL665	20,000 tests	400 µg	61FG2XLB
Anti-HA XL665	5,000 tests	100 µg	610HAXLA
Anti-HA XL665	20,000 tests	400 µg	610HAXLB
Anti-DNP XL665	5,000 tests	100 µg	61DNPXLA
Anti-DNP XL665	20,000 tests	400 µg	61DNPXLB

Anti-immunoglobulins	Size	Active moiety per vial (average)	Cat#
Anti-mouse IgG-XL665	5,000 tests	100 µg	61PAMXLA
Anti-mouse IgG-XL665	20,000 tests	400g	61PAMXLB
Anti-rabbit IgG-XL665	5,000 tests	100 µg	61PARXLA
Anti-rabbit IgG-XL665	20,000 tests	400 µg	61PARXLB
Anti-human IgG-XL665	5,000 tests	100 µg	61HFCXLA
Anti-human IgG-XL665	20,000 tests	400 µg	61HFCXLB

Affinity sytem reagents	Size	Active moiety per vial (average)	Cat#
Streptavidin XL665	5,000 tests	250 µg	610SAXLA
Streptavidin XL665	20,000 tests	1,000 µg	610SAXLB
Streptavidin XL <sup>entl</sup>	5,000 tests	250 µg	611SAXLA
Streptavidin XL <sup>entl</sup>	20,000 tests	1,000 µg	611SAXLB
WGA XL665	5,000 tests	100 µg	61WGAXLA*
WGA XL665	20,000 tests	400 µg	61WGAXLB *
ConA XL665	5,000 tests	500 µg	61CNAXLA *
ConA XL665	20,000 tests	2,000 µg	61CNAXLB *
Protein A XL665	5,000 tests	50 µg	61PRAXLA *
Protein A XL665	20,000 tests	200 µg	61PRAXLB *

\* On request

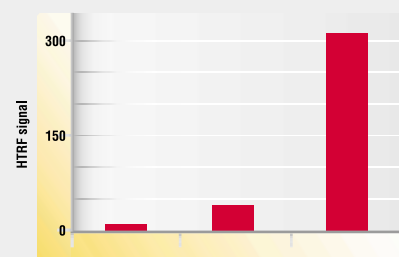
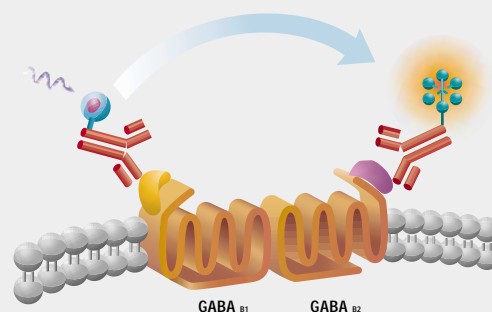
## HTRF<sup>®</sup> reagent toolbox

### ASSAY IN ACTION

Maurel et al. have recently demonstrated how HTRF<sup>®</sup> could be applied to investigating receptor dimerization, which plays a major role in G-protein activation.

Using anti-HA and anti-c-myc reagents from the HTRF<sup>®</sup> toolbox, and two tagged subunits of  $\gamma$ -aminobutyric acid type B (GABA<sub>B</sub>) receptor, they were able to monitor the subunit association and thereby demonstrate the usefulness of these assay formats for studying protein:protein interactions at the membrane level. This shows how the HA and c-myc tagged subunits are able to associate and how this interaction can be detected using HTRF<sup>®</sup> reagents. Liu et al. further illustrated the use of HTRF<sup>®</sup> in a recent publication describing the mechanisms underlying GABA<sub>B</sub> receptor allosteric modulation.

From Maurel D. et al. *Anal Biochem.* 2004;329:253-62.



HA-GABA <sub>B1</sub> -ASA	-	+	+
myc-V2	-	+	-
myc-GABA <sub>B2</sub>	-	-	+

### SELECTED BIBLIOGRAPHY

- Liu J, Maurel D, Etzol S, Brabet I, Ansanay H, Pin JP, Rondard P. Molecular determinants involved in the allosteric control of agonist affinity of the GABA<sub>A</sub> receptor by the GABA<sub>B2</sub> subunit. *J Biol Chem.* 2004;279:15824-30.
- Maurel D, Kniazeff J, Mathis G, Trinquet E, Pin JP, Ansanay H. Cell surface detection of membrane protein interaction with homogeneous time-resolved fluorescence resonance energy transfer technology. *Anal Biochem.* 2004;329:253-62.
- Amoravain M, Ouled-Diaf J, Pelorjas L, Sulocha S, Courtin O, Parize M, Lopez-Crapez E, Seguin P. Streptavidin-XL<sup>™</sup>, an ideal tool for the detection of biological targets using HTRF<sup>®</sup> technology. Miptec, Basel, May 2003.
- Leblanc V, Delaunay V, Lelong JC, Gas F, Mathis G, Grassi J, May E. Homogeneous Time-Resolved Fluorescence assay for identifying p53 interactions with its protein partners, directly in a cellular extract. *Anal Biochem.* 2002;308:247-54.
- Préaudat M, Ouled-Diaf J, Alpha-Bazin B, Mathis G, Mitsugi T, Aono Y, Takahashi K, Takemoto H. A homogeneous caspase 3 activity assay using HTRF<sup>®</sup> technology. *J Biomol Screening.* 2002;7:267-74.
- Kane SA, Fleener CA, Zhang YS, Davis LJ, Musselman AL, Huang PS. Development of a binding assay for p53/HDM2 by using homogeneous time-resolved fluorescence. *Anal Biochem.* 2000;278:29-38.
- Mellor GW, Burden MN, Préaudat M, Joseph Y, Cooksley SB, Ellis JH, Banks MN. Development of a CD28/CD86 (B7-2) binding assay for high throughput screening by homogeneous time-resolved fluorescence. *J Biomol Screening.* 1998;3:91-9.
- Zhou G, Cummings R, Li Y, Mitra S, Wilkinson HA, Eibrecht A, Hermes JD, Schaeffer

This poster can be downloaded from [www.htrf.com](http://www.htrf.com)

- JM, Smith RG, Moller DE. Nuclear receptors have distinct affinities for coactivators: characterization by fluorescence resonance energy transfer. *Mol Endocrinol.* 1998;12:1594-604.
- Slostra JW, Kuperus D, Pluckthun A, Meloen RH. Identification of new tag sequences with differential and selective recognition properties for the anti-FLAG<sup>®</sup> monoclonal antibodies M1, M2 and M5. *Mol Divers.* 1996;2:156-64.
- Mathis G. Probing Molecular Interactions with Homogeneous Techniques Based on Rare Earth cryptates and Fluorescence Energy Transfer. *Clin. Chem.* 1995;41/9:1391-7.
- Prat O, Lopez E, Mathis G. Detection of europium cryptates by time-resolved fluorescence. In *Non-isotopic Probing, Blotting, and Sequencing*. Ed Academic Press. 1995: 307-29.
- Le Doussal JM, Gautherot E, Martin M, Barbet J, Delaage M. Enhanced in vivo targeting of an asymmetric bivalent hapten to double-antigen-positive mouse B cells with monoclonal antibody conjugate cocktails. *J Immunol.* 1991;146:169-75.
- Kolodziej PA, Young RA. Epitope tagging and protein surveillance. *Meth Enzymol.* 1991;194:508-19.
- Hochuli E. Purification of recombinant proteins with metal chelate adsorbent. *Gene Eng.* (NY). 1990;12:87-98.
- Evan GI, Lewis GK, Ramsay G, Bishop JM. Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product. *Mol Cell Biol.* 1985;5:3610-6.

