



Toolbox reagents



Anti-tag conjugates
Anti-species
Affinity reagents

HTRF[®] reagent toolbox

HTRF[®] 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[®], and HA. Small organic motifs like biotin and dinitrophenyl (DNP) are also widely used for developing assays. The HTRF[®] 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 Eu³⁺ cryptate and to XL665. The SA-XL^{entl} 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 [®]	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

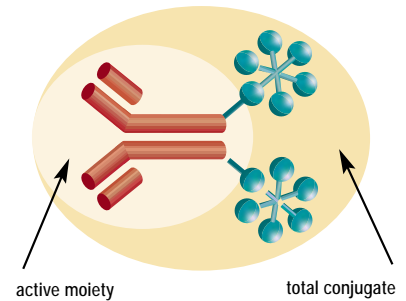
- A unique set of reagents for developing homogeneous high throughput assays.
- Allows flexible and straightforward scaling up for primary and secondary screening phases.
- Enables multiple target developments and assay configurations, such as protein:protein and nuclear receptor screening, receptor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

HTRF[®] reagent toolbox

Principle of use and definitions

All reagents in the HTRF[®] toolbox are supplied with reference to a 384 low volume well format, for a final assay volume of 20 μ L. HTRF[®] 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:



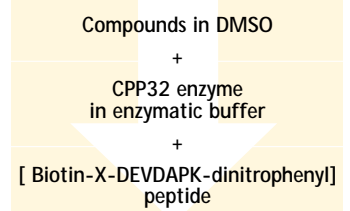
For instance, antibodies conjugated to XL665 are supplied on the basis of 20 ng of antibody per well. However, their equivalence in terms of average conjugate quantity per well depends on the molar ratio antibody/XL665, and therefore varies from one toolbox reagent to another. In practice, the active 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 Eu³⁺ 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 Eu³⁺ cryptate and XL665 conjugate:

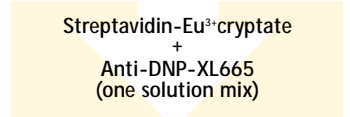
Most assays can be run within the nanomolar range. However, as a tracer, Eu³⁺ cryptate conjugates must not be excessive in order to prevent reader saturation and an unacceptable level of background. In most cases, an Eu³⁺ cryptate concentration of 4 to 5 nM is appropriate, and will typically generate 620 nm fluorescence of about 40,000 cps on RUBYstar, the HTRF[®] reference reader. As an example, for an antibody conjugated to Eu³⁺ 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[®] 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[®] 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.

ENZYMATIC STEP: 90 MIN



DETECTION STEP: 60 MIN



READ

HTRF® reagent toolbox

Eu³⁺ cryptate conjugates

All Eu³⁺ 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 ^{entl}	5,000 tests	250 µg	611SAXLA
Streptavidin XL ^{entl}	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

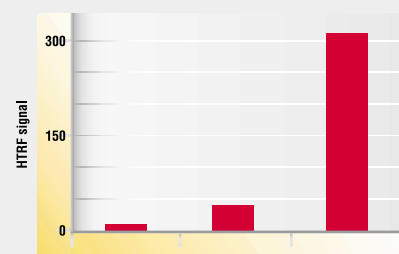
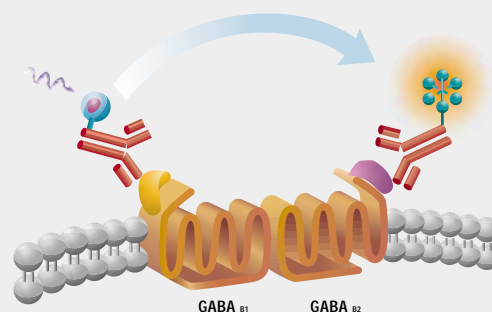
HTRF[®] reagent toolbox

ASSAY IN ACTION

Maurel et al. have recently demonstrated how HTRF[®] 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[®] toolbox, and two tagged subunits of γ -aminobutyric acid type B (GABA_B) 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[®] reagents. Liu et al. further illustrated the use of HTRF[®] in a recent publication describing the mechanisms underlying GABA_B receptor allosteric modulation.

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



HA-GABA _{B1} -ASA	-	+	+
myc-V2	-	+	-
myc-GABA _{B2}	-	-	+

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This poster can be downloaded from www.htrf.com

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