

R&D, Administration and Europe Office

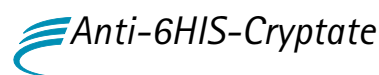
Cisbio Bioassays
 Phone: +33 (0)4 66 79 67 05
 Fax: +33 (0)4 66 79 19 20
 E-mail: bioassays@cisbio.com

Japan Office

Sceti Medical Labo K.K.
 Phone: +81 (0)3 5510 2932
 Fax: +81 (0)3 5510 0130
 E-mail: reagent@scetimedilabo.co.jp

USA Office

Cisbio US, Inc.
 Phone : 888 963 4567
 Fax : 781 687 1500
 E-mail : htrfinfo@cisbio.us



Eu³⁺ Cryptate-conjugated mouse monoclonal antibody anti-6 Histidine

For in vitro research use only
 Storage temperature : 2-8°C

www.htrf.com

HTRF® package insert

Document reference : 61HISKLA/B rev08 (July 2009)

Packaging details :

| | 384-well low volume plate (20 µl) |
|----------|-----------------------------------|
| 61HISKLA | 5,000 tests |
| 61HISKLB | 20,000 tests |

1. Background

The development of fusion protein technology has boosted the use of toolbox reagents for the purification and the detection of recombinant proteins. This technique consists in the addition of a specific sequence (i.e. tag) to the protein to be expressed. These tags can be inserted at different places in the sequence and are often added to N or C-terminal ends to guarantee the production of a biologically active recombinant protein. The protein can then be detected through the tag using toolbox reagents (e.g. antibodies raised against this tag or proteins having an affinity for it).

6HIS tag – six consecutive histidine amino acids – is a widely used tag in molecular biology. Both eukaryotic and bacterial expression vectors for the production of 6HIS tagged proteins are commercially available. In most cases the 6HIS tag does not interfere with the structure or the function of the recombinant protein (enzymes,...). 6HIS motif also allows the purification of the protein on metal chelating solid phases.

Mouse monoclonal antibody HIS-1 is an IgG2a which was raised against a polyhistidine tagged fusion protein. It recognizes native as well as denatured – reduced forms of synthetic polyhistidine or polyhistidine-tagged fusion protein. The product is known as reactive with fusion protein expressed by prokaryotic pET, pRSET and pTrc expression vectors. This monoclonal is suitable for use in a wide variety of biomolecular interactions such as protein:protein binding or receptor:ligand binding applications.

The accessibility of the 6HIS motif on the fusion proteins by anti-6HIS toolbox reagents can be assessed using the HTRF® 6HIS check kit (62HISPEB).

This toolbox reagent has been developed for High Throughput Screening using the HTRF technology. HTRF is an homogeneous time-resolved fluorescent technique, based on the energy transfer between a long-life fluorescent cryptate donor (Europium or Lumi4-Terbium) and HTRF acceptors such as XL665, d2, or other suitable acceptor fluorophores (i.e. GFP, fluorescein...). The transferred energy is then emitted as detectable fluorescent signal. In HTRF assays, the donor and the acceptor are conjugated to biomolecules (anti-tag antibodies, streptavidins, peptides,...) for studying molecular interactions.

References

Trinquet E. Studying molecular interactions with the new Lumi4®-Tb Cryptate HTRF toolbox. SBS 15th annual conference 2009, Lille (France).

Hochuli E. Purification of recombinant proteins with metal chelate adsorbent. Gene Eng.(NY). 1990;12:87-98.

Mathis G. Probing molecular interactions with homogeneous techniques based on rare earth cryptates and fluorescence energy transfer. Clin Chem. 1995;41:1391-7

Porath J, Carlsson J, Olsson I, and Belfrage G. Metal chelate affinity chromatography, a new approach to protein fractionation. Nature. 1975;258:598-9.

2. Reagent description

HIS-1 monoclonal antibody is produced by Sigma and is labeled with Eu³⁺ Cryptate at Cisbio Bioassays. Specific activity of the conjugate ranges from 4 to 6 Cryptates/antibody.

This conjugate is lyophilized in 100 mM Phosphate pH 7.0, 0.5% protease free bovine serum albumin (BSA) and stabilizers.

The concentration of this conjugate has been calibrated in order to obtain a 620 nm signal within the 30,000-50,000 count range. This calibration was run in a 384-well low volume plate format using a final volume of 20 µL, a buffer containing 0.1% BSA and 0.4 M KF, and a PHERAstar Plus reader (BMG LABTECH). A recommended amount of antibody per well is specified in the product description sheet attached to the product. On this basis, each vial from the two available sizes enables the assessment of 5,000 and 20,000 tests respectively. Actual amount per well will be dependent on optimized assay conditions.

3. Reagent handling

3.1. Preparation of the working solution

- Allow the lyophilized reagent to warm up at room temperature for at least 30 minutes.
- For the 5,000 test vial (61HISKLA) : reconstitute the product with 250 µL of distilled water.
- For the 20,000 test vial (61HISKLB) : reconstitute the product with 1 mL of distilled water.
- Vortex the solution gently.
- After reconstitution, the stock solution can be divided into aliquots and frozen at -20°C for additional use (according to storage conditions, see §5).
- Dilute with buffer the stock solution to the working concentration. Mix gently

3.2. Recommended buffer

Most common buffers can be used for the preparation of the working solution, providing that the pH is maintained between 5.5 and 8.5. They can be complemented with BSA (0.1%) to prevent reagent coating, and detergents such as Tween 20, Triton X100, CHAPS (up to 0.5%)... may also be added. Avoid SDS, due to its denaturing effect on XL665. It is recommended to check the background signal by counting the buffer blank.

KF can play an essential role in the lanthanide cryptate protection by preventing the action of possible quenchers contained in the assay. **KF is mandatory for HTRF assays using Europium cryptate.** KF is generally used at a final concentration of 100 to 400mM, and is added to the conjugate working solutions or dispensed in a separate step, just before the readout. Assay using **Lumi4-Tb cryptate donor, does not require KF.**

4. Assay flexibility and miniaturization

When used as suggested, one vial from the two available sizes will provide sufficient reagent for 5,000 and 20,000 tests respectively using a 384-well low volume plate in 20 μ L final assay volume (HTRF[®] packaged basis).

To move to other plate formats (96 half-well or 1536-well) and final volumes (100 μ L to less than 10 μ L), the volume of each assay component is simply proportionally adjusted in order to maintain the reagent concentrations as for the 20 μ L final assay volume. For instance, in the case of the 1536-well format in 10 μ L final volume, 2 times less material per well is used, thereby allowing 10,000 and 40,000 tests respectively to be run. The performances of the HTRF[®] assay remain the same whatever the level of miniaturization.

| Assay components | Volume proportion | Assay format | | |
|------------------------|-------------------|------------------------|----------------------------------|----------------------------|
| | | 1536-well (10 μ L) | 384-well low volume (20 μ L) | 96 half-well (100 μ L) |
| Other assay components | 2 volume | 5 μ L | 10 μ L | 50 μ L |
| Acceptor conjugate | 1 volume | 2.5 μ L | 5 μ L | 25 μ L |
| Cryptate conjugate | 1 volume | 2.5 μ L | 5 μ L | 25 μ L |
| | Small size | 10,000 tests | 5,000 tests | 1,000 tests |
| | Bulk size | 40,000 tests | 20,000 tests | 4,000 tests |

Plate references : 96 half-well plate (Costar # 3694 or equivalent), 384-well low volume plate (Greiner # 784076), 1536-well (Greiner # 782086).

5. Storage conditions and stability

Lyophilized anti-6HIS-Cryptate conjugate should be stored at 2-8°C until reconstituted. Under proper storing conditions, this reagent is stable until the expiry date indicated on the product description sheet.

Once reconstituted, stock solutions are stable one day at 2-8°C. They can be refrozen (at -20°C) and thawed once only. Do not repeat freezing and thawing.

Caution ! It is possible to combine donor and acceptor conjugates immediately before use. Do not store donor and acceptor conjugates mixed together for extended periods of time.