

#### 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

Sctei Medical Labo K.K.  
Phone : +81-3-5510-2932  
Fax : +81-3-5510-0130  
E-mail: reagent@sctimedilabo.co.jp

#### USA Office

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



 **Anti FLAG® (M2)-Tb**

**Lumi4®-Tb Cryptate-conjugated mouse monoclonal antibody Anti-FLAG®**

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

[www.htrf.com](http://www.htrf.com)

## HTRF® package insert

Document reference : 61FG2TLA/B rev00 (July 2009)

#### Packaging details :

	384-well low volume plate (20 µL)
61FG2TLA	5,000 tests
61FG2TLB	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 generic reagents (e.g. antibodies raised against this tag or proteins having an affinity for it).

Chimeric constructs with the hydrophilic octapeptide FLAG® epitope have been widely used as a multi-purpose tag for the identification, detection, and purification of FLAG® fusion proteins both in procaryotes and in eukaryotic cell lines. Commercially available vectors enable the expression of recombinant proteins whose tag includes the FLAG® sequence.

Anti-FLAG®M2 is a purified IgG1 monoclonal antibody isolated from a Murine cell culture that binds to FLAG® fusion proteins. Unlike anti-FLAG®M1 antibody the M2 antibody will recognize the FLAG® sequence at the N-terminus, Met-N-terminus or C-terminus of FLAG® fusion proteins. M2 antibody is useful for identification of FLAG® fusion proteins by common immunological procedures. It is suitable for use in a wide variety of protein-protein binding and receptor-ligand binding. M2 binding is not calcium dependent.

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)
- Knappik A, Pluckthun A. An improved affinity tag base on the FLAG® peptide for the detection and purification of recombinant antibody fragments. *Biotechniques* 1994;17(4):754-61
- Slootstra JW, Kuperus D, Pluckthun A, Meloen RH. Identification of new tag sequences with differential and selective recognition properties for the anti-FLAG® monoclonal antibodies M1, M2 and M5. *Mol Divers* 1996;2(3):156-64
- Mathis G. Probing molecular interactions with homogeneous techniques based on rare earth cryptates and fluorescence energy transfer. *Clin Chem*. 1995;41:1391-7

## 2. Reagent description

M2 monoclonal antibody is produced by Sigma-Aldrich and is labeled with Lumi4®-Tb Cryptate at Cisbio Bioassays. Specific activity of the conjugate ranges from 4 to 8 Cryptates/antibody.

This conjugate is lyophilized in 100 mM Phosphate pH 7.0, 0.1% protease free bovine serum albumin (BSA) and stabilizers.

The concentration of this conjugate has been calibrated in order to obtain a good sensitivity in HTRF assays. 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 0.4M KF, and a PHERAstar Plus (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 (61FG2TLA) : reconstitute the product with 250 µL of distilled water .
- For the 20,000 test vial (61FG2TLB) : 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 µL final assay volume (HTRF® packaged basis).

To move to other plate formats (96 half-well or 1536-well) and final volumes (100 µL to less than 10 µL), the volume of each assay component is simply proportionally adjusted in order to maintain the reagent concentrations as for the 20 µL final assay volume. For instance, in the case of the 1536-well format in 10 µL 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 µL)	384-well low volume (20 µL)	96 half-well (100 µL)
Other assay components	2 volume	5 µL	10 µL	50 µL
Acceptor conjugate	1 volume	2.5 µL	5 µL	25 µL
Cryptate conjugate	1 volume	2.5 µL	5 µL	25 µL
	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-FLAG®-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 2 days 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.**