

R&D, Administration and Europe Office

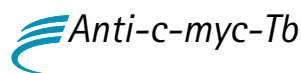
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Lumi4®-Tb Cryptate-conjugated mouse monoclonal antibody anti-c-myc

For in vitro research use only
 Storage temperature : -20°C

www.htrf.com

HTRF® package insert

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

Packaging details :

	384-well low volume plate (20 µL)
61MYCTAA	5,000 tests
61MYCTAB	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).

Proto-oncogene c-myc (the human homologue of v-myc gene) encodes a polypeptide with an apparent molecular weight of 62 KDa. The c-myc epitope tag derived from this sequence is widely used in both eukaryotic and bacterial expression systems. Commercially available vectors enable the expression of recombinant proteins whose tag includes the minimum c-myc 410-419 sequence (i.e. the anti-c-myc antibody epitope). The insertion of this motif allows the detection and the immunopurification of c-myc-tagged fusion proteins.

Mouse monoclonal antibody 9E10 is an IgG1 which was raised against a synthetic peptide corresponding to the 408-439 sequence of human c-myc protein. It recognizes the EQKLISEEDL motif and is specific for human c-myc although a slight cross-reaction has been observed with murine c-myc at high antibody concentration. This monoclonal was shown to react with c-myc-tagged fusion proteins from various origins. It is suitable for use in a wide variety of protein-protein binding and receptor-ligand binding applications.

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).
- Evan GI, Lewis GK, Ramsay G and Bishop JM. Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product. *Mol Cell Biol.* 1985;5:3610-6.
- Field J, Nikawa JJ, Broek D et al. Purification of RAS responsive adenyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. *Mol Cell Biol.* 1988;8:2159-65.
- Kolodziej PA and Young RA. Epitope tagging and protein surveillance. *Methods Enzymol.* 1991;194:508-19.
- 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

9E10 monoclonal antibody is labeled with Lumi4®-Tb Cryptate at Cisbio Bioassays. Specific activity of the conjugate ranges from 4 to 8 Cryptates/antibody.

This conjugate is frozen in 100 mM phosphate pH 7.0, 0.1% protease free bovine serum albumin (BSA) and 0.1% Tween.

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-low volume plate format using a final volume of 20 µL, a buffer containing 0.1% BSA, 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 each vial of frozen conjugate (61MYCTAA and 61MYCTAB) to thaw at room temperature.
- Mix gently.
- Dilute each stock solution of conjugate with buffer to the working concentration.
- Mix gently.
- Unused stock solution of conjugates can be divided into aliquots and frozen at -20°C for additional use (according to storage conditions, see §5).

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

Frozen anti-c-myc-Tb conjugate should be stored at -20°C. Under proper storing conditions, this reagent is stable until the expiry date indicated on the product description sheet.

Once thawed, stock solutions are stable for two 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.