

Japan Office

USA Office



XL665-conjugated Rabbit anti-Mouse IgG

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

www.htrf.com

HTRF® package insert

Document reference : 61PAMXLA/B rev02 (July 2008)

Packaging details :

	384-well low volume plate (20 µL)
61PAMXLA	5,000 tests
61PAMXLB	20,000 tests

1. Background

Anti-species antibodies may be used to detect the binding of unlabeled specific antibody on a target molecule. They may also be used to detect Ig fusion proteins.

Anti-species antibodies provide a useful alternative to custom labeling or less specific immunobinding proteins such as protein A. Polyclonal antibodies should be affinity purified against the antigen for best results.

This reagent has been carefully selected in order to minimize cross reactions with human proteins and other animal serum proteins. Nevertheless, it is always recommended to control the appropriate blank levels in all types of assays.

This toolbox reagent has been developed for High Throughput Screening using the HTRF®. HTRF® is an homogeneous time-resolved fluorescence technique, based on the non-radiative energy transfer of the long-lived emission from the Europium Cryptate donor to the cross-linked allophycocyanin (XL665) acceptor.

Reference

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

IgGs from Rabbit anti-Mouse immunoserum have been isolated by immunoaffinity chromatography using Mouse IgG coupled to agarose beads. The purified polyclonal antibodies were labeled with XL665. Specific activity of the conjugate ranges from 1.2 to 1.6 XL665 per antibody.

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

In 384-well low volume plate format using a final volume of 20 µL, each vial from the two available sizes enables respectively the assessment of 5,000 and 20,000 tests on the basis of 20 ng of antibody per well (i.e. 0.13 pmol/well). Actual concentration per well will be dependent on optimized assay conditions. As a general rule, anti-Mouse IgG-XL665 working concentration should never be lower than that of the tagged compound to be detected.

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 (61PAMXLA) : reconstitute the product with 250 µL of distilled water in order to obtain a 0.4 mg/mL solution (antibody concentration).
- For the 20,000 test vial (61PAMXLB) : reconstitute the product with 1 mL of distilled water in order to obtain a 0.4 mg/mL solution (antibody concentration).
- Vortex the solution gently.
- After reconstitution, the stock solution can be divided into aliquots and frozen for additional use (according to storage conditions, see §5).
- Dilute 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.

Potassium fluoride (KF) at a final concentration of 100 to 400 mM must be used for HTRF®. Optimal concentration must be determined experimentally. KF plays an essential role in trisbipyridine europium cryptate protection by preventing the action of possible quenchers contained in the assay at the time of fluorescence measurement. KF can be added to the conjugate working solutions. However, **the presence of KF during all phases of the assay is NOT mandatory** since all potential quenching processes with europium cryptate are reversible. **KF is only required during fluorescence readout** and it may therefore be dispensed in a separate step, just before counting.

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
XL665 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-mouse IgG-XL665 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 label.

Once reconstituted, stock solutions are stable two weeks at 2-8°C. They can be refrozen (at -80°C) and thawed at least two more times.

Caution ! It is possible to combine Cryptate and XL665 conjugates immediately before use. Do not store Cryptate and XL665 conjugates mixed together for extended periods of time.