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Biotin conjugated Poly-(Glu, Ala, Tyr) (6:3:1)

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

www.htrf.com

HTRF® package insert

Document reference : 61GATBLA/B rev07 (July 2008)

Packaging details :

	384-well low volume plate (20 µL)
61GATBLA	5,000 tests
61GATBLB	20,000 tests

1. Background

Poly-(Glu,Ala,Tyr) (6:3:1) is a random copolymer which was shown to be a substrate for tyrosine-specific protein kinases.

Since tyrosine is the only residue that can form a stable phosphorylated product, the polymer can be used as substrate even in crude homogenates without the complications of a large phosphorylation background by the abundant serine-specific protein kinases. The use of polymeric substrates is often an economical solution to enzyme specific substrates such as src gene peptides.

Poly-(GAT)-biotin may be used with Streptavidin-XL665 and Anti-phosphotyrosine-cryptate for easily optimized HTRF® tyrosine kinase assays.

This toolbox reagent has been developed for High Throughput Screening using the HTRF® technology. 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.

References

Braun S, Raymond WE and Racker E. Synthetic tyrosine polymers as substrates and inhibitors of tyrosine-specific protein kinases. J Biol Chem. 1984;259:2051-4

Kolb AJ, Kaplita PV, and Hayes DJ. Tyrosine kinase assays adapted to homogeneous time-resolved fluorescence. Drug Discovery Today. 1998;3:333-42

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

Poly-(GAT)-biotin is produced at Cisbio international. This conjugate is lyophilized in 20 mM Veronal pH 7.0, 0.05% protease free bovine serum albumin (BSA).

In 384-well low volume plate format, using a final volume of 20 µL per well, each vial from the two available sizes enables respectively the assessment of 5,000 and 20,000 tests on the basis of 50 ng of Poly-(GAT) per well (mean M.W.=25 kDa, i.e. 2 pmol/well). Actual concentration 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 (61GATBLA) : reconstitute the product with 250 µL of distilled water in order to obtain a 1 mg/mL solution (conjugate concentration).
- For the 20,000 test vial (61GATBLB) : reconstitute the product with 1 mL of distilled water in order to obtain a 1 mg/mL solution (conjugate 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 poly-(GAT)-biotin 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 2 weeks at 2-8°C. They can be refrozen (at -20°C) and thawed at least two more times.