

MANUAL

Technology: HTRF®

Toolbox

Mab Anti Flag-Tb

Part number	61FGBTLF	61FGBTLA	61FGBTLB
Test size	1,000 tests	5,000 tests	20,000 tests

Storage: 2-8°C

Assay volume: 20 µL

Version: 01

Date: January 2024

REAGENT DESCRIPTION

In an HTRF protein/protein interaction assay, one protein is labeled (directly or indirectly) with the donor, and the other protein is labeled (directly or indirectly) with the acceptor. When the two proteins interact, the donor molecule is brought into proximity with the acceptor molecule. Excitation of the donor will result in signal generation proportional to the binding of proteins.

Monoclonal Anti-Flag antibody was labeled with Lumi4® Tb. The antibody will recognize the FLAG® sequence at the N-terminus, intra or C-terminus of FLAG® fusion proteins.

MATERIALS

REAGENT	1,000 TESTS	5,000 TESTS	20,000 TESTS
MAB Anti Flag-Tb Lyophilized In phosphate buffer pH 7.0 containing protease free bovine serum albumin.	1 vial - 0,25 mL	1 vial - 0,25 mL	1 vial - 1 mL

REVVITY REAGENTS NOT PROVIDED	PART#
PPI - Terbium detection buffer	Cat # 61DB10RDF* *200 mL - ready-to-use
Plates - HTRF 96-well low volume plate	66PL96001

For HTRF microplate recommendations, and for a list of HTRF® - compatible readers and set-up recommendations, please visit our website.

For reading, an HTRF® - compatible reader is needed. Make sure to use the appropriate set-up.

STORAGE AND STABILITY

- Store the reagent at 2-8°C.
- Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.
- Once thawed, stock solutions are stable for two days at 2-8°C. They can be refrozen (at ≤ -16°C) and thawed once only. Do not repeat freezing and thawing.

ASSAY FORMAT

When used as suggested, one vial from the three available sizes will provide sufficient reagent for 1,000; 5,000 and 20,000 tests respectively, using a 20 µL final assay volume.

	VOLUME
Other assay components	10 µL
Acceptor (d2 or XL665) conjugate	5 µL
Donor (Terbium) conjugate	5 µL
Final volume*	20 µL

*Assay volumes can be adjusted proportionally to run the assay in 96- or 1536-well microplates.

REAGENT HANDLING

Buffers

Revivity PPI - Terbium detection buffer Cat # 61DB10RDF* has been optimized for maximum performance.

It is mandatory to use the same buffer to prepare the donor and the acceptor (d2 or XL665) conjugates.

When using specific in-house buffers for the preparation of working solutions, we recommend a basic buffer such as PBS (Phosphate Buffered Saline) or Hepes with a pH maintained between 5.5 and 8.5.

It can be supplemented with BSA (0.1%), and detergents such as Tween 20, Triton X100 or CHAPS (up to 0.5%) to prevent reagent coating. Avoid SDS, due to its denaturing effect on XL665.

Please note that a phosphate-free buffer must be used in biochemical kinase assays to prevent interferences pertaining to the binding of anti-phospho specific antibodies.

Conjugates

Allow each vial conjugate to warm up at room temperature. Reconstitute the lyophilizate following the instructions below:

Mix gently.

MAB ANTI MBP-EU CRYPTATE	STOCK SOLUTION PREPARATION	WORKING SOLUTION PREPARATION (SEE ASSAY FORMAT ABOVE)
1,000 tests	0,25 mL Mix gently	Dilute 20-fold the stock solution in PPI detection buffer Mix gently E.g. Add 4.75 mL PPI detection buffer to 0.25 mL of stock solution
5,000 tests	0,25 mL Mix gently	Dilute 100-fold the stock solution in PPI detection buffer Mix gently E.g. Add 24.75 mL PPI detection buffer to 0.25 mL of stock solution
20,000 tests	1 mL Mix gently	Dilute 100-fold the stock solution in PPI detection buffer Mix gently E.g. Add 99 mL PPI detection buffer to 1 mL of stock solution

Additional info is included in the batch information provided with the reagent.

The optimal amount per well will be dependent on assay conditions. For additional information on assay optimization, please refer to the technical note: Guidelines for optimizing protein: protein interaction assays using HTRF PPI reagents.

Make sure to prepare stock and working solutions according to the instructions that correspond to the packaging you have purchased (number of tests).

This product contains material of biological origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage.

REACH European regulations and compliance. This product and/or some of its components include a Triton concentration of 0.1% or more and as such, it is concerned by the REACH European regulations. We recommend researchers using this product to act in compliance with REACH and in particular: to only use the product for in vitro research in appropriate and controlled premises by qualified researchers, ii) to ensure the collection and the treatment of subsequent waste, and iii) to make sure that the total amount of Triton handled does not exceed 1 ton per year.



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www.revvity.com

revvity

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

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