

# revvity

# MAb Anti FLAG M2-Tb cryptate

Part # 61FG2TLF, 61FG2TLA & 61FG2TLB

Test size: 1,000 tests (61FG2TLF) - 5,000 tests (61FG2TLA) - 20,000 tests (61FG2TLB) Assay volume: 20 µL Revision: #04 of September 2023 Store at 2-8°C For research use only. Not for use in therapeutic or diagnostic procedures.

# **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.

Mouse monoclonal antibody labeled with Lumi4<sup>®</sup> Tb is an IgG1 raised against FLAG<sup>®</sup> fusion proteins. Unlike anti-FLAG<sup>®</sup>M1 antibody, the M2 antibody will recognize the FLAG<sup>®</sup> sequence at the N-terminus or C-terminus of FLAG<sup>®</sup> fusion proteins.

### MATERIALS

Reagent	1,000 tests	5,000 tests	20,000 tests
MAb Anti FLAG M2-Tb cryptate Lyophilized In phosphate buffer pH 7.0 containing protease free bovine serum albumin.	1 vial	1 vial	1 vial

Revvity reagents Not provided	Part #
PPI - Terbium detection buffer 200 mL - ready-to-use	Cat # 61DB10RDF*
Plates* - HTRF 96-well low volume plate	66PL96001

\*For HTRF microplate recommendations, please visit www.revvity.com

For reading, an HTRF<sup>®</sup>-compatible reader is needed. Make sure to use the appropriate setup.

For a list of HTRF®-compatible readers and setup recommendations, please visit www.revvity.com

## 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 reconstituted, 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 two available sizes will provide sufficient reagent for 1,000 tests, 5,000 tests and 20,000 tests respectively, using a 20 µL final assay volume. Assay volumes can be adjusted proportionally to run the assay in 96- or 1536-well microplates.

	Volume
Other assay components	10 µL
Acceptor (d2 or XL665) conjugate	5 µL
Donor (Eu or Tb Cryptate) conjugate	5 µL
Final volume*	20 µL

#### **REAGENT HANDLING**

#### **BUFFERS**

Revvity 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.

Use of Tb cryptate donor does not require KF

#### **CONJUGATES**

Allow each vial of lyophilized conjugate to warm up at room temperature.

Reconstitute the lyophilisate following the following instructions:

MAb Anti FLAG M2-Tb cryptate*	Stock solution preparation	Working solution preparation (see assay format above)
	Add 0.25 mL of distilled water	Dilute 20-fold the stock solution in PPI detection buffer
1,000 tests	Mix gently	Mix gently
		E.g. Add 4.75 mL PPI detection buffer to 0.25 mL of stock solution
	Add 0.25 mL of distilled water	Dilute 100-fold the stock solution in PPI detection buffer
5,000 tests	Mix gently	Mix gently
		E.g. Add 24.75 mL PPI detection buffer to 0.25 mL of stock solution
	Add 1 mL of distilled water	Dilute 100-fold the stock solution in PPI detection buffer
20,000 tests	Mix gently	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).

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