MANUAL

Technology: HTRF™ Protein-Protein Interaction

Mab Anti Flag-d2

Part number	61FGBDLF	61FGBDLA	61FGBDLB
Test size	1,000 tests	5,000 tests	20,000 tests

Storage: 2-8°C

Assay volume : 20 µL

Version: 02 **Date:** July 2024

For research only. Not for use in 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.

Monoclonal Anti-Flag antibody was labelled with d2. 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-d2 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 PPI - Europium detection buffer PPI - Low Ionic Strength Europium detection buffer 220 mL - ready-to-use	Cat # 61DB10RDF Cat # 61DB9RDF Cat # 61DB11RDF
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 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.

	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**

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

REAGENT HANDLING

Buffers

Revvity PPI - Terbium detection buffer Cat # 61DB10RDF, Revvity PPI - Europium detection buffer Cat # 61DB9RDF and Revvity PPI - Low Ionic Strength Eu detection buffer Cat # 61DB11RDF have been optimized for maximum performance. The buffer of choice is selected based on the donor type and sensitivity of the PPI interaction to ionic strength.

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 Europium toolbox conjugates requires a final KF concentration between 100 mM and 400 mM.
Use of Terbium conjugates does not require KF.

Conjugates

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

Reconstitute the lyophilizate following the following instructions:

Mab Anti Flag-d2	Stock solution preparation	Working solution preparation (see assay format above)
1,000 tests	Add 0.25 mL of distilled water. 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	Add 0.25 mL of distilled water. 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	Add 1 mL of distilled water. 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).

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