



MAb Anti HA-d2

PROTOCOL

Part # 610HADAF, 610HADAA & 610HADAB

Test size: 1,000 tests (610HADAF), 5,000 tests (610HADAA), 20,000 tests (610HADAB)

Assay volume: 20 μ L

Revision: 03 (May 2018)

Store at $\leq -16^{\circ}\text{C}$

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 d2 is an IgG2b raised against the twelve-amino acid peptide sequence CYPYDVPDYASL. It recognizes the influenza virus hemagglutinin epitope (YPYDVPDYA) which has been used extensively as a general epitope tag in expression vectors.

MATERIALS

Reagent	1,000 tests	5,000 tests	20,000 tests
MAb Anti HA-d2 Frozen in phosphate buffer pH 7.0 containing protease free bovine serum albumin	1 vial - 0.08 mL	1 vial - 0,4 mL	1 vial - 1,6 mL

Cisbio reagents Not provided	Part #
PPI - Terbium detection buffer PPI - Europium detection buffer	Cat # 61DB10RDF* Cat # 61DB9RDF* *200 mL - ready-to-use
Plates - HTRF 96-well low volume plate	66PL96001

For HTRF microplate recommendations, please visit <http://www.cisbio.com/microplate-recommendations>

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

For a list of HTRF[®] - compatible readers and set-up recommendations, please visit <http://www.cisbio.com/readers>

STORAGE AND STABILITY

- Store the reagent at $\leq -16^{\circ}\text{C}$.
- 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 $^{\circ}\text{C}$. They can be refrozen (at $\leq -16^{\circ}\text{C}$) 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

Cisbio PPI - Terbium detection buffer Cat # 61DB10RDF* and Cisbio PPI - Europium detection buffer Cat # 61DB9RDF* have been optimized for maximum performance. The buffer of choice is selected based on the donor type in the assay.

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 frozen conjugate to warm up at room temperature.

Mix gently.

MAB Anti HA-d2	Stock solution preparation	Working solution preparation (see assay format above)
1,000 tests	0.08 mL Mix gently	Dilute 62,5-fold the stock solution in PPI detection buffer Mix gently E.g. Add 4,92 mL PPI detection buffer to 0.08 mL of stock solution
5,000 tests	0,4 mL Mix gently	Dilute 62,5-fold the stock solution in PPI detection buffer Mix gently E.g. Add 24.6 mL PPI detection buffer to 0.4 mL of stock solution
20,000 tests	1,6 mL Mix gently	Dilute 62,5-fold the stock solution in PPI detection buffer Mix gently E.g. Add 98.4 mL PPI detection buffer to 1,6 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.

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