

MANUAL

Technology: HTRF™

Protein-Protein Interaction

PAb Anti Mouse IgG-Eu cryptate

Part number	61PAMKLA	61PAMKLB
Test size	5,000 tests	20,000 tests

Storage: 2-8°C

Assay volume : 20 µL

Version: 07

Date: July 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.

IgGs from Rabbit anti-Mouse immunoserum were isolated by immunoaffinity chromatography using Mouse IgG coupled to agarose beads. The purified polyclonal antibodies have been labeled with Eu³⁺ cryptate. This reagent may be used to detect the binding of unlabeled mouse specific antibody on a target molecule or on Ig fusion proteins.

This reagent may cross-react with immunoglobulins from other species. Therefore, it is always recommended to control the appropriate blanks levels in all types of assays specially when IgG from other animal species are involved.

MATERIALS

REAGENT	5,000 TESTS	20,000 TESTS
PAb Anti Mouse IgG-Eu cryptate Lyophilized In phosphate buffer pH 7.0 containing protease free bovine serum albumin.	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 5,000 tests and 20,000 tests respectively, using a 20 μ L final assay volume.

VOLUME	
Other assay components	10 μ L
Acceptor (d2 or XL665) conjugate	5 μ L
Donor (Eu or Tb Cryptate) conjugate	5 μ L
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.

Conjugates

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

PAb Anti Mouse IgG-Eu cryptate	Stock solution preparation	Working solution preparation (see assay format above)
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).

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.



The information provided in this document is for reference purposes only and may not be all-inclusive. Revvity, Inc., its subsidiaries, and/or affiliates (collectively, "Revvity") do not assume liability for the accuracy or completeness of the information contained herein. Users should exercise caution when handling materials as they may present unknown hazards. Revvity shall not be liable for any damages or losses resulting from handling or contact with the product, as Revvity cannot control actual methods, volumes, or conditions of use. Users are responsible for ensuring the product's suitability for their specific application. REVVITY EXPRESSLY DISCLAIMS ALL WARRANTIES, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, REGARDLESS OF WHETHER ORAL OR WRITTEN, EXPRESS OR IMPLIED, ALLEGEDLY ARISING FROM ANY USAGE OF ANY TRADE OR ANY COURSE OF DEALING, IN CONNECTION WITH THE USE OF INFORMATION CONTAINED HEREIN OR THE PRODUCT ITSELF

Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet - FRANCE

www.revvity.com

revvity

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

(800) 762-4000
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com
Copyright ©2023, Revvity, Inc. All rights reserved.