

# MANUAL

Technology: HTRF™

Protein-Protein Interaction

## MAb Anti GST-XL665

Part number	61GSTXLF	61GSTXLA	61GSTXLB
Test size	1,000 tests	5,000 tests	20,000 tests

Storage: 2-8°C

Assay volume : 20 µL

Version: 11

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.

Mouse monoclonal antibody labeled with XL665 is an IgG2a raised against Glutathione S-transferase. This antibody has been shown to react with GST-tagged fusion proteins from a large number of expressing vectors.

## MATERIALS

REAGENT	1,000 TESTS	5,000 TESTS	20,000 TESTS
MAb Anti GST-XL665 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. 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
<b>Final volume*</b>	<b>20 µL</b>

\*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 GST-XL665	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).

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](http://www.revvity.com)

revvity

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

(800) 762-4000  
[www.revvity.com](http://www.revvity.com)

For a complete listing of our global offices, visit [www.revvity.com](http://www.revvity.com)  
Copyright ©2023, Revvity, Inc. All rights reserved.