

HYBRIDOMA BINDING (GST ANTIGEN) KITS

Part # 64HYGSTPEG & 64HYGSTPEH

Test size: 500 tests (64HYGSTPEG), 10 000 tests (64HYGSTPEH) - assay volume: 20 μ L

Revision: #02 of September 2023

Store at: $\leq -60^{\circ}\text{C}$

This product is intended for research purposes only. The product is not intended to be used for therapeutic or diagnostic purposes.

ASSAY PRINCIPLE

This kit is intended for the simple and rapid screening of mouse hybridoma clones from cell supernatant.

The detection principle of this kit is based on HTRF[®] technology (Homogeneous Time-Resolved Fluorescence).

As shown in Figure 1, Mouse hybridoma binding for a GST-tagged antigen is measured using a sandwich immunoassay involving an anti GST antibody labeled with Europium (HTRF donor) and an anti mouse antibody labeled with d2 (HTRF acceptor). When the donor and acceptor antibodies are brought into close proximity due to mouse hybridoma binding, excitation of the donor antibody triggers fluorescence resonance energy transfer (FRET) towards the acceptor antibody, which in turn emits specifically at 665 nm. This specific signal is directly proportional to the binding of a mouse hybridoma clone to its GST-tagged target antigen.

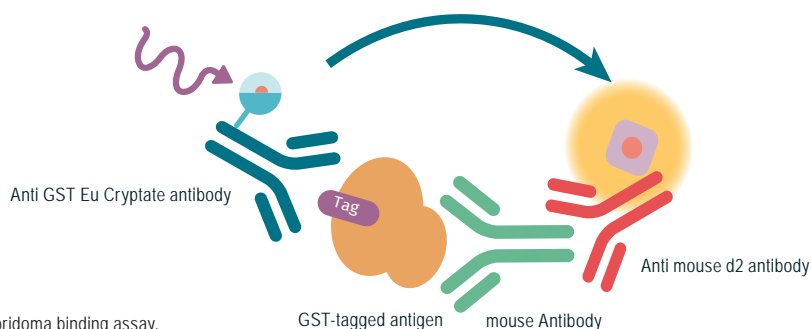
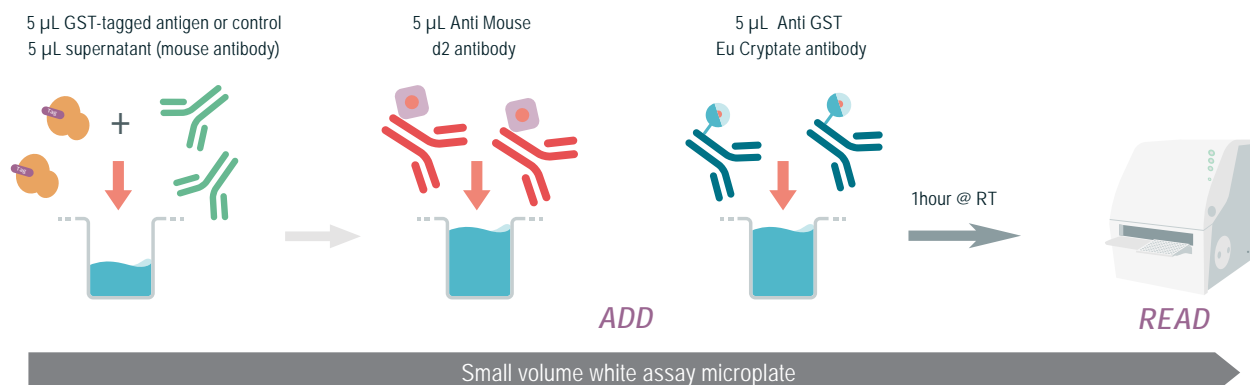


Figure 1: Principle of the HTRF hybridoma binding assay.

MANUAL AT A GLANCE



MATERIALS:

KIT COMPONENTS	500 TESTS CAT # 64HYGSTPEG	10,000 TESTS CAT # 64HYGSTPEH
Control Frozen	1 vial	1 vial
Anti GST Eu Cryptate antibody Frozen	1 vial - 100 μ L 25X	1 vial - 2 mL 25X
Anti Mouse d2 antibody Frozen	1 vial - 100 μ L 25X	1 vial - 2 mL 25X
PPI Europium Detection Buffer Frozen	1 vial - 20 mL	1 vial - 220 mL

For reading, an HTRF[®]-Certified Reader is needed. Make sure to use the set-up for Eu Cryptate. For a list of HTRF-compatible readers and setup recommendations, please visit our website at: www.revvity.com

For HTRF microplate recommendations, please visit: www.revvity.com

STORAGE AND STABILITY

Store the kit at $\leq -60^{\circ}\text{C}$. Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.

To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at $\leq -16^{\circ}\text{C}$.









Thawed PPI Europium Detection Buffer can be stored at $2-8^{\circ}\text{C}$ on your premises.

REAGENT PREPARATION**BEFORE YOU BEGIN:**

- It is very important to prepare reagents in the specified PPI Europium detection buffer. The use of an incorrect buffer may affect reagent stability and assay results.
- Before use, allow all reagents to warm up to room temperature then homogenize buffer. It is recommended to filter buffer before use.
- The HTRF detection solutions must be prepared in individual vials and can be premixed prior to dispensing.

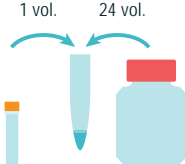
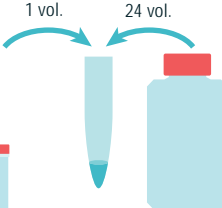
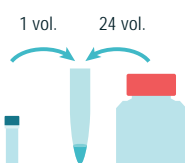
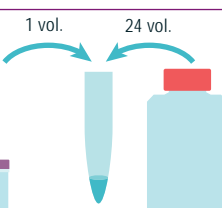
TO PREPARE STOCK SOLUTIONS:

Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.






500 TESTS		10,000 TESTS	
Control*			
Thaw the Control. Mix gently. This ready-to-use control can be frozen and stored at $\leq -60^{\circ}\text{C}$.			Thaw the Control. Mix gently. This ready-to-use control can be frozen and stored at $\leq -60^{\circ}\text{C}$.
*The control in the hybridoma binding kit is only provided as an internal assay control to check the quality of the results obtained. The window between control and negative control should be greater than 2.			
Anti GST Eu Cryptate antibody			
Thaw the Anti GST Eu Cryptate antibody Mix gently. This 25X Anti GST Eu Cryptate antibody stock solution can be frozen and stored at $\leq -16^{\circ}\text{C}$.			Thaw the Anti GST Eu Cryptate antibody Mix gently. This 25X Anti GST Eu Cryptate antibody stock solution can be frozen and stored at $\leq -16^{\circ}\text{C}$.
Anti mouse d2 antibody			
Thaw the Anti mouse d2 antibody. Mix gently. This 25X Anti mouse d2 antibody stock solution can be frozen and stored at $\leq -16^{\circ}\text{C}$.			Thaw the Anti mouse d2 antibody. Mix gently. This 25X Anti mouse d2 antibody stock solution can be frozen and stored at $\leq -16^{\circ}\text{C}$.
PPI Europium Detection Buffer			
Thaw the PPI Europium Detection Buffer The thawed buffer can be stored at $2-8^{\circ}\text{C}$ on your premises.			Thaw the PPI Europium Detection Buffer The thawed buffer can be stored at $2-8^{\circ}\text{C}$ on your premises..

TO PREPARE ANTI GST EU CRYPTATE ANTIBODY AND ANTI MOUSE D2 ANTIBODY WORKING SOLUTIONS:

Each well requires 5 μL of each donor & acceptor antibodies.

500 TESTS		10,000 TESTS	
Anti GST Eu Cryptate antibody			
Dilute 25-fold the 25X stock solution (thawed reagent) of Anti GST Eu Cryptate antibody with PPI Europium Detection Buffer : e.g. 0.1 mL of thawed Anti GST Eu Cryptate antibody stock solution + 2.4 mL of PPI Europium Detection Buffer.			Dilute 25-fold the 25X stock solution (thawed reagent) of Anti GST Eu Cryptate antibody with PPI Europium Detection Buffer : e.g. 2 mL of thawed Anti GST Eu Cryptate antibody stock solution + 48 mL of PPI Europium Detection Buffer.
Anti mouse d2 antibody			
Dilute 25-fold the 25X stock solution (thawed reagent) of Anti mouse d2 antibody with PPI Europium Detection Buffer : e.g. 0.1 mL of thawed Anti mouse d2 antibody stock solution + 2.4 mL of PPI Europium Detection Buffer.			Dilute 25-fold the 25X stock solution (thawed reagent) of Anti mouse d2 antibody with PPI Europium Detection Buffer : e.g. 2 mL of thawed Anti mouse d2 antibody stock solution + 48 mL of PPI Europium Detection Buffer.

ASSAY MANUAL

		Control*	Samples
STEP 1		Dispense into each standard well 10 µL of control	Dispense into each sample well 5 µL of GST-tagged antigen 5 µL of supernatant
STEP 2		Dispense into all control & sample wells 5 µL of Anti mouse d2 antibody	
STEP 3		Dispense into all control & sample wells 5 µL of Anti GST Eu Cryptate antibody	
STEP 4		Seal the plate and incubate for 1 hour at room temperature	
STEP 5		Remove the plate sealer and read on an HTRF® compatible reader.	

*The control in the hybridoma binding kit is only provided as an internal assay control to check the quality of the results obtained. The window between control and negative control should be greater than 2.

STANDARD MANUAL FOR ASSAY IN 20 µL FINAL VOLUME

	Control*	GST-tagged antigen	Cell supernatant	Anti GST Eu Cryptate antibody	Anti mouse d2 antibody	PPI Europium detection buffer
Negative control	-	5 µL	-	5 µL	-	10 µL
Buffer control	-	-	-	-	-	20 µL
Positive control - Control*	10 µL	-	-	5 µL	5 µL	-
Sample	-	5 µL	5 µL	5 µL	5 µL	-

DATA REDUCTION & INTERPRETATION

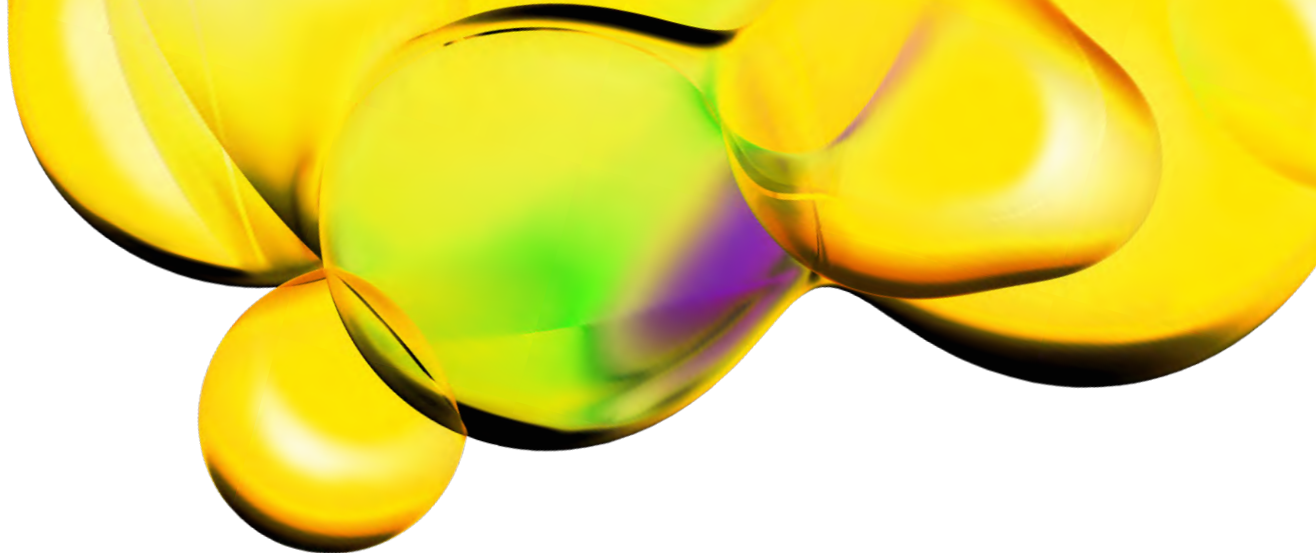
1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit www.revvity.com



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

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