

## HYBRIDOMA BINDING (HIS ANTIGEN) KITS

Part # 64HYHISPEG & 64HYHISPEH

Test size: 500 tests (64HYHISPEG), 10 000 tests (64HYHISPEH) - assay volume: 20  $\mu$ 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<sup>®</sup> technology (Homogeneous Time-Resolved Fluorescence).

As shown in Figure 1, Mouse hybridoma binding for a HIS-tagged antigen is measured using a sandwich immunoassay involving an anti HIS 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 HIS-tagged target antigen.

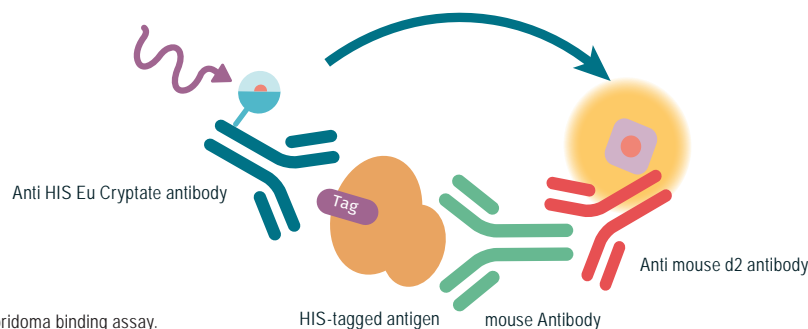
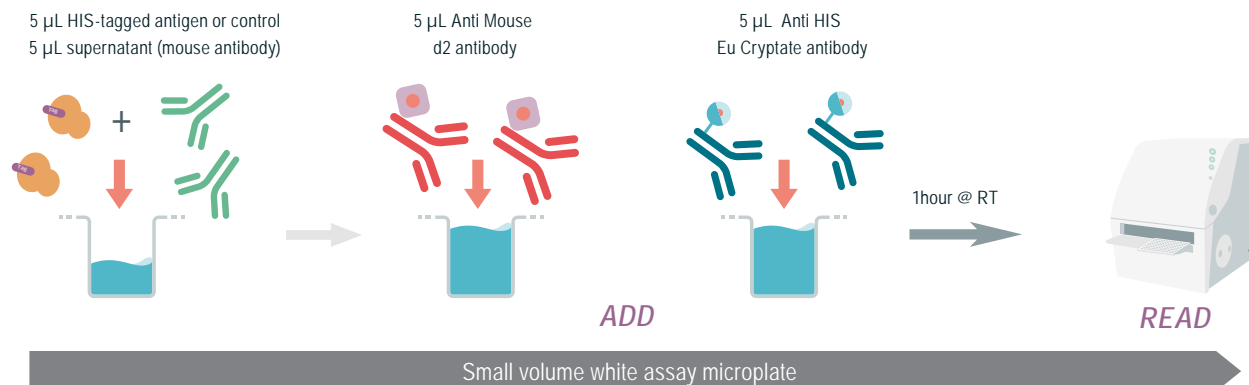


Figure 1: Principle of the HTRF hybridoma binding assay.

### MANUAL AT A GLANCE



**MATERIALS:**

| <b>KIT COMPONENTS</b>                | <b>500 TESTS<br/>CAT # 64HYHISPEG</b> | <b>10,000 TESTS<br/>CAT # 64HYHISPEH</b> |
|--------------------------------------|---------------------------------------|--|
| Control Frozen                       | 1 vial                                | 1 vial                                   |
| Anti HIS Eu Cryptate antibody Frozen | 1 vial - 100 $\mu$ L<br>25X           | 1 vial - 2 mL<br>25X                     |
| Anti Mouse d2 antibody Frozen        | 1 vial - 100 $\mu$ L<br>25X           | 1 vial - 2 mL<br>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.revivity.com](http://www.revivity.com)

For HTRF microplate recommendations, please visit: [www.revivity.com](http://www.revivity.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.

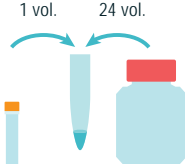
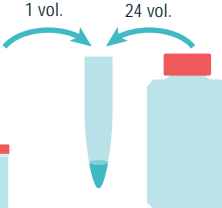
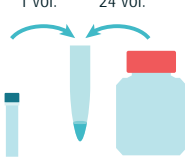
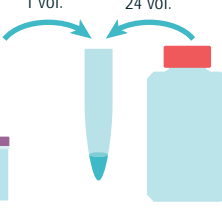
## TO PREPARE STOCK SOLUTIONS:

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.<br>Mix gently.<br>This ready-to-use control can be frozen and stored at $\leq -60^{\circ}\text{C}$ .  |    |   | Thaw the Control.<br>Mix gently.<br>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 HIS Eu Cryptate antibody   |   |  |   |
| Thaw the Anti HIS Eu Cryptate antibody<br>Mix gently.<br>This 25X Anti HIS Eu Cryptate antibody stock solution can be frozen and stored at $\leq -16^{\circ}\text{C}$ .   |    |   | Thaw the Anti HIS Eu Cryptate antibody<br>Mix gently.<br>This 25X Anti HIS 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.<br>Mix gently.<br>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.<br>Mix gently.<br>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<br>The thawed buffer can be stored at $2-8^{\circ}\text{C}$ on your premises.  |  |  | Thaw the PPI Europium Detection Buffer<br>The thawed buffer can be stored at $2-8^{\circ}\text{C}$ on your premises..   |

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

Each well requires 5  $\mu\text{L}$  of each donor & acceptor antibodies.

| 500 TESTS   |   | 10,000 TESTS   |  |
|---|---|--|--|
| Anti HIS Eu Cryptate antibody   |   |  |  |
| Dilute 25-fold the 25X stock solution (thawed reagent) of Anti HIS Eu Cryptate antibody with PPI Europium Detection Buffer :<br>e.g. 0.1 mL of thawed Anti HIS Eu Cryptate antibody stock solution + 2.4 mL of PPI Europium Detection Buffer. |  |  | Dilute 25-fold the 25X stock solution (thawed reagent) of Anti HIS Eu Cryptate antibody with PPI Europium Detection Buffer :<br>e.g. 2 mL of thawed Anti HIS 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 :<br>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 :<br>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<br>5 µL of HIS-tagged antigen<br>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 HIS 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* | HIS-tagged antigen | Cell supernatant | Anti HIS 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 &amp; INTERPRETATION

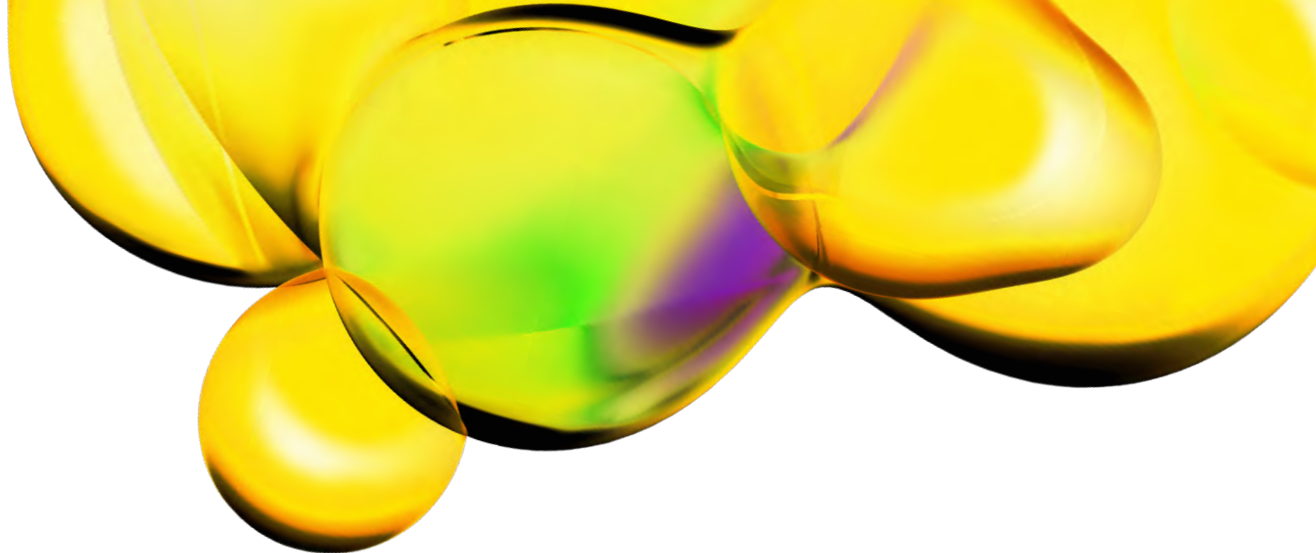
1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

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

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



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