



## IL6/IL6R BINDING ASSAY KITS

Part # 64BDIL6PEG & 64BDIL6PEH

Test size: 500 tests (64BDIL6PEG), 10 000 tests (64BDIL6PEH) - 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

The HTRF IL6/IL6R Binding Assay is designed to measure the interaction between IL6 and IL6R. Utilizing HTRF (Homogeneous Time-resolved Fluorescence) technology, the assay enables simple and rapid characterization of compound and antibody blockers in a high throughput format.

As shown in Figure 1, the interaction between IL6 and IL6R is detected by using anti-Tag1 labeled with Europium (HTRF donor) and anti-Tag2 labeled with XL665 (HTRF acceptor). When the donor and acceptor antibodies are brought into close proximity due to IL6 and IL6R 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 extent of IL6/IL6R interaction. Thus, compound or antibody blocking IL6/IL6R interaction will cause a reduction in HTRF signal.

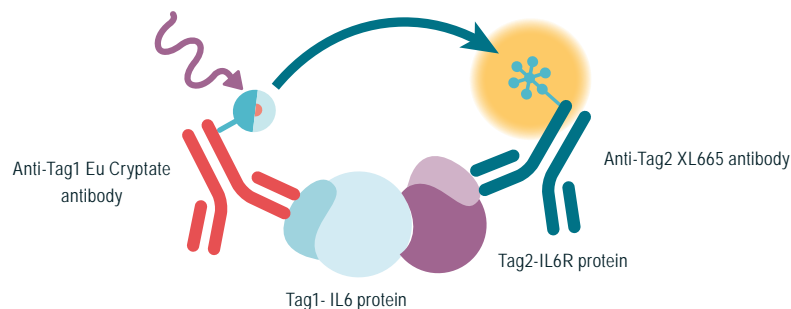
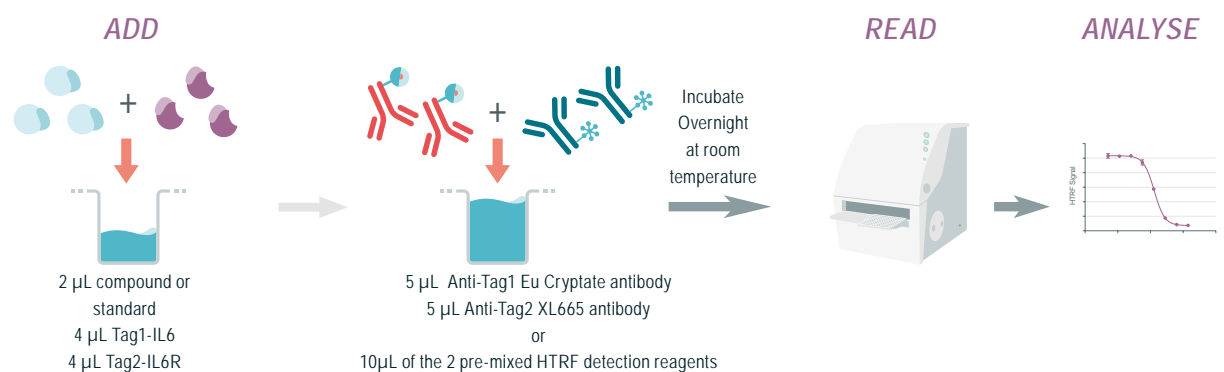


Figure 1: Principle of the HTRF IL6/IL6R assay.

### MANUAL AT A GLANCE



Small volume white assay microplate

**MATERIALS:**

KIT COMPONENTS	500 TESTS CAT # 64BDIL6PEG	10,000 TESTS CAT # 64BDIL6PEH
Tag1-IL6 Lyophilized	1 vial	2 vials
Tag2-IL6R Lyophilized	1 vial	2 vials
IL6-IL6R standard Frozen	1 vial - 40 $\mu$ L 4 $\mu$ M	1 vial - 40 $\mu$ L 4 $\mu$ M
Anti-Tag1 Eu Cryptate antibody- Frozen	1 vial - 50 $\mu$ L 50X	1 vial - 1 mL 50X
Anti-Tag2 XL665 antibody Frozen	1 vial - 50 $\mu$ L 50X	1 vial - 1 mL 50X
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.revvy.com](http://www.revvy.com)

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

Once reconstituted, tagged IL6 & IL6R stock solution may be frozen, and can be thawed only once. Once thawed (or reconstituted), anti-Tag solutions can be frozen once.

To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at  $\leq -60^{\circ}\text{C}$ .













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.
- Thaw the frozen reagents at room temperature.
- Before use, allow all reagents to warm up to room temperature then homogenize buffer. It is recommended to filter buffers before use.
- The tagged protein solutions must be prepared in individual vials - DO NOT premix tagged solutions prior to dispensing.
- The anti-Tag solutions must be prepared in individual vials and can be premixed prior to dispensing.
- Compounds may be prepared in PPI Europium detection buffer.

## 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	
Tag1-IL6			
Reconstitute the Tag1-IL6 with 200 $\mu$ L of distilled water in order to obtain a 10X stock solution. Mix gently, DO NOT vortex ! This stock solution can be frozen and stored at $\leq 60^{\circ}\text{C}$ .			Reconstitute the Tag1-IL6 with 2 mL of distilled water in order to obtain a 10X stock solution. Mix gently, DO NOT vortex ! This stock solution can be frozen and stored at $\leq 60^{\circ}\text{C}$ .
Tag2-IL6R			
Reconstitute the Tag2-IL6R with 200 $\mu$ L of distilled water in order to obtain a 10X stock solution. Mix gently, DO NOT vortex ! This stock solution can be frozen and stored at $\leq 60^{\circ}\text{C}$ .			Reconstitute the Tag2-IL6R with 2 mL of distilled water in order to obtain a 10X stock solution. Mix gently, DO NOT vortex ! This stock solution can be frozen and stored at $\leq 60^{\circ}\text{C}$ .
IL6-IL6R Standard			
Thaw the IL6-IL6R standard. Mix gently. This 5X standard stock solution can be frozen and stored at $\leq 60^{\circ}\text{C}$ .			Thaw the IL6-IL6R standard. Mix gently. This 5X standard stock solution can be frozen and stored at $\leq 60^{\circ}\text{C}$ .
Anti-Tag1 Eu Cryptate antibody			
Thaw the Anti-Tag1 Eu Cryptate antibody. Mix gently. This 50X Anti-Tag1 Eu Cryptate antibody stock solution can be frozen and stored at $\leq 60^{\circ}\text{C}$ .			Thaw the Anti-Tag1 Eu Cryptate antibody. Mix gently. This 50X Anti-Tag1 Eu Cryptate antibody stock solution can be frozen and stored at $\leq 60^{\circ}\text{C}$ .
Anti-Tag2 XL665 antibody			
Thaw the Anti-Tag2 XL665 antibody. Mix gently. This 50X Anti-Tag2 XL665 antibody stock solution can be frozen and stored at $\leq 60^{\circ}\text{C}$ .			Thaw the Anti-Tag2 XL665 antibody. Mix gently. This 50X Anti-Tag2 XL665 antibody stock solution can be frozen and stored at $\leq 60^{\circ}\text{C}$ .
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 TAG1-IL6 AND TAG2-IL6R WORKING SOLUTIONS:

Each well requires 4  $\mu$ L of each Tag-protein.

500 TESTS		10,000 TESTS	
Tag1-IL6			
Dilute 10-fold the 10X stock solution (reconstituted reagent) of Tag1-IL6 with PPI Europium Detection Buffer : e.g. 200 $\mu$ L of reconstituted Tag1-IL6 stock solution + 1800 $\mu$ L of PPI Europium Detection Buffer .			Dilute 10-fold the 10X stock solution (reconstituted reagent) of Tag1-IL6 with PPI Europium Detection Buffer : e.g. 2 mL of reconstituted Tag1-IL6 stock solution + 18 mL of PPI Europium Detection Buffer .
Tag2-IL6R			
Dilute 10-fold the 10X stock solution (reconstituted reagent) of Tag2-IL6R with PPI Europium Detection Buffer : e.g. 200 $\mu$ L of reconstituted Tag2-IL6R stock solution + 1800 $\mu$ L of PPI Europium Detection Buffer .			Dilute 10-fold the 10X stock solution (reconstituted reagent) of Tag2-IL6R with PPI Europium Detection Buffer : e.g. 2 mL of reconstituted Tag2-IL6R stock solution + 18 mL of PPI Europium Detection Buffer .

## TO PREPARE ANTI-TAG1 EU CRYPTATE ANTIBODY AND ANTI-TAG2 XL665 ANTIBODY WORKING SOLUTIONS:

Each well requires 5  $\mu$ L of each anti-Tag donor & acceptor reagents.

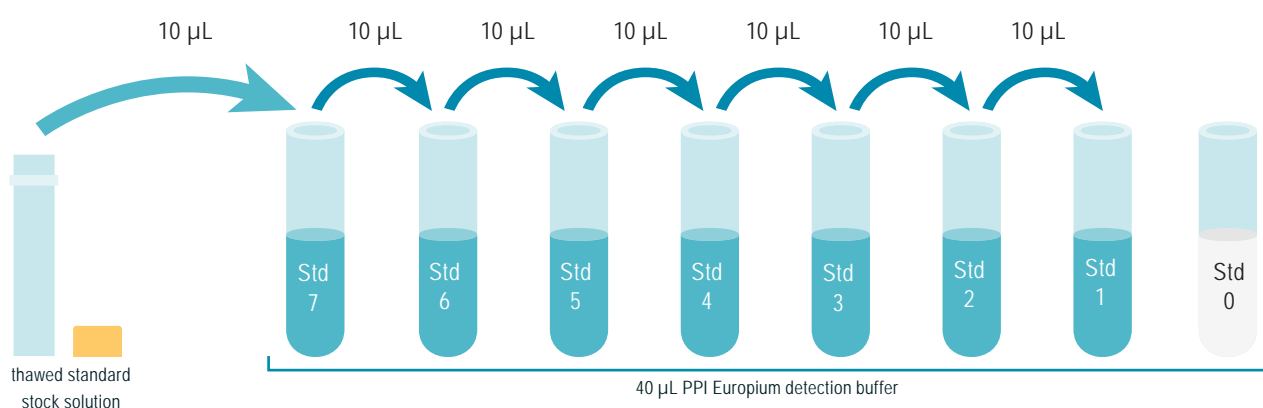
500 TESTS		10,000 TESTS	
Anti-Tag1 Eu Cryptate antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of Anti-Tag1 Eu Cryptate antibody with PPI Europium Detection Buffer : e.g. 50 $\mu$ L of reconstituted Anti-Tag1 Eu Cryptate antibody stock solution + 2450 $\mu$ L of PPI Europium Detection Buffer .			Dilute 50-fold the 50X stock solution (thawed reagent) of Anti-Tag1 Eu Cryptate antibody with PPI Europium Detection Buffer : e.g. 1 mL of reconstituted Anti-Tag1 Eu Cryptate antibody stock solution + 49 mL of PPI Europium Detection Buffer .
Anti-Tag2 XL665 antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of Anti-Tag2 XL665 antibody with PPI Europium Detection Buffer : e.g. 50 $\mu$ L of reconstituted Anti-Tag2 XL665 antibody stock solution + 2450 $\mu$ L of PPI Europium Detection Buffer .			Dilute 50-fold the 50X stock solution (thawed reagent) of Anti-Tag2 XL665 antibody with PPI Europium Detection Buffer : e.g. 1 mL of reconstituted Anti-Tag2 XL665 antibody stock solution + 49 mL of PPI Europium Detection Buffer .
anti-Tag HTRF detection solutions (pre-mixed)			
Pre-mix the two ready-to-use anti-Tag HTRF detection solutions just prior to dispensing the reagents: e.g. 2.5 mL of Anti-Tag1 Eu Cryptate antibody + 2.5 mL of Anti-Tag2 XL665 antibody			Pre-mix the two ready-to-use anti-Tag HTRF detection solutions just prior to dispensing the reagents: e.g. 20 mL of Anti-Tag1 Eu Cryptate antibody + 20 mL of Anti-Tag2 XL665 antibody

## TO PREPARE WORKING IL6-IL6R STANDARD SOLUTIONS:

- Each well requires 2  $\mu\text{L}$  of standard.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.





A recommended standard dilution procedure is listed and illustrated below:

1. Thaw the standard vial, the concentration of the IL6-IL6R standard stock solution = 4  $\mu\text{M}$  (4 000 000 pM)
  2. Prepare the following dilutions:
    - Dilute the thawed standard stock solution 5-fold with PPI Europium detection buffer.  
In practice: take 10  $\mu\text{L}$  of stock solution and add it to 40  $\mu\text{L}$  of PPI Europium detection buffer. Mix gently. This yields the high standard (Std 7: 800 000 pM) for the top of the curve.
    - Use the high standard (Std 7) to prepare the standard curve using 5-fold serial dilutions as follows:
      - Dispense 40  $\mu\text{L}$  of PPI Europium detection buffer into each vial from Std 6 to Std 0
      - Add 10  $\mu\text{L}$  of standard to 40  $\mu\text{L}$  of PPI Europium detection buffer, mix gently and repeat the serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.
- This will create 7 standards for the analyte. Std 0 is PPI Europium detection buffer alone.



STANDARD	SERIAL DILUTIONS	WORKING SOLUTIONS	FINAL CONCENTRATIONS
Standard Stock solution	Thaw the IL6-IL6R standard stock solution	4 $\mu\text{M}$ (4 000 000 pM)	
Standard 7	10 $\mu\text{L}$ standard stock solution + 40 $\mu\text{L}$ PPI Europium detection buffer	800 000 pM	80 000 pM
Standard 6	10 $\mu\text{L}$ Standard 7 + 40 $\mu\text{L}$ PPI Europium detection buffer	160 000 pM	16 000 pM
Standard 5	10 $\mu\text{L}$ Standard 6 + 40 $\mu\text{L}$ PPI Europium detection buffer	32 000 pM	3200 pM
Standard 4	10 $\mu\text{L}$ Standard 5 + 40 $\mu\text{L}$ PPI Europium detection buffer	6400 pM	640 pM
Standard 3	10 $\mu\text{L}$ Standard 4 + 40 $\mu\text{L}$ PPI Europium detection buffer	1280 pM	128 pM
Standard 2	10 $\mu\text{L}$ Standard 3 + 40 $\mu\text{L}$ PPI Europium detection buffer	256 pM	25.6 pM
Standard 1	10 $\mu\text{L}$ Standard 2 + 40 $\mu\text{L}$ PPI Europium detection buffer	51.2 pM	5.12 pM
Standard 0	40 $\mu\text{L}$ PPI Europium detection buffer	0 pM	0 pM

## ASSAY MANUAL

		Standard	Samples
<b>STEP 1</b>		Dispense into each standard well 2 $\mu$ L of standard 4 $\mu$ L of Tag1-IL6 4 $\mu$ L of Tag2-IL6R.	Dispense into each sample well 2 $\mu$ L of compound/antibody or buffer 4 $\mu$ L of Tag1-IL6 4 $\mu$ L of Tag2-IL6R.
<b>STEP 2</b>		Dispense into all standard & sample wells 10 $\mu$ L of pre-mixed Anti-Tag1 Eu Cryptate antibody and Anti-Tag2 XL665 antibody	
<b>STEP 3</b>		Seal the plate and incubate for Overnight.at room temperature	
<b>STEP 4</b>		Remove the plate sealer and read on an HTRF® compatible reader.	

### STANDARD MANUAL FOR INHIBITORY ASSAY IN 20 $\mu$ L FINAL VOLUME

	Standard	Inhibitor	Tag1-IL6	Tag2-IL6R	Anti-Tag1 Eu Cryptate antibody	Anti-Tag2 XL665 antibody	PPI Europium detection buffer
Standard	2 $\mu$ L	-	4 $\mu$ L	4 $\mu$ L	5 $\mu$ L	5 $\mu$ L	-
Sample	-	2 $\mu$ L	4 $\mu$ L	4 $\mu$ L	5 $\mu$ L	5 $\mu$ L	-
Positive control	-	-	4 $\mu$ L	4 $\mu$ L	5 $\mu$ L	5 $\mu$ L	2 $\mu$ L
Negative control	-	-	4 $\mu$ L	-	5 $\mu$ L	5 $\mu$ L	6 $\mu$ L
Buffer control	-	-	-	-	-	-	20 $\mu$ L

## EXAMPLE OF PLATE MAP

	1	2	3	4	5	6
A	Buffer control: 20 µL PPI Europium detection buffer	Repeat Well A1	Repeat Well A1	Compound...: 2 µL compound... 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well A4	Repeat Well A4
B	Negative control: 6 µL PPI Europium detection buffer 4 µL Tag1-IL6 10 µL pre-mix anti-Tag reagents	Repeat Well B1	Repeat Well B1	Compound...: 2 µL compound... 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well B4	Repeat Well B4
C	Positive control: 2 µL PPI Europium detection buffer 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well C1	Repeat Well C1	Compound...: 2 µL compound... 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well C4	Repeat Well C4
D	Std 0: 2 µL Standard 0 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well D1	Repeat Well D1	Compound...: 2 µL compound... 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well D4	Repeat Well D4
E	Std 1: 2 µL Standard 1 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well E1	Repeat Well E1	Compound...: 2 µL compound... 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well E4	Repeat Well E4
F	Std 2: 2 µL Standard 2 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well F1	Repeat Well F1	Compound...: 2 µL compound... 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well F4	Repeat Well F4
G	Std 3: 2 µL Standard 3 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well G1	Repeat Well G1	Compound...: 2 µL compound... 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well G4	Repeat Well G4
H	Std 4: 2 µL Standard 4 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well H1	Repeat Well H1			
I	Std 5: 2 µL Standard 5 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well I1	Repeat Well I1			
J	Std 6: 2 µL Standard 6 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well J1	Repeat Well J1			
K	Std 7: 2 µL Standard 7 4 µL Tag1-IL6 4 µL Tag2-IL6R 10 µL pre-mix anti-Tag reagents	Repeat Well K1	Repeat Well K1			

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

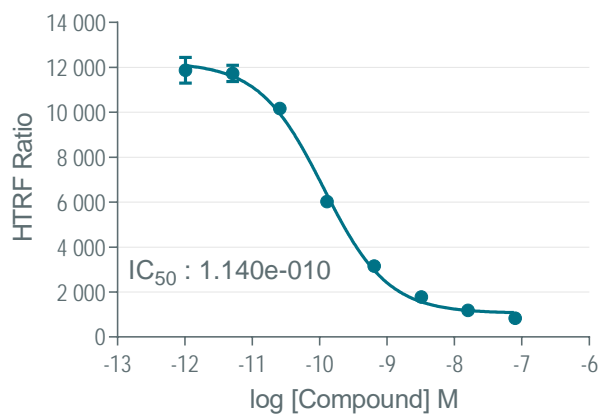
## RESULTS

The data shown below must not be substituted for the data obtained in the laboratory, and should be considered only as an example.

Readouts on HTRF® compatible reader.

Note that results may vary from one HTRF® compatible reader to another.

Standard curve







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