MANUAL

# IL17A/IL17RA BINDING ASSAY KITS

Part # 64BDIL17PEG & 64BDIL17PEH

Test size: 500 tests (64BDIL17PEG), 10 000 tests (64BDIL17PEH) - assay volume: 20 µL

Revision: #02 of September 2023

Store at: ≤-60°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 IL17A/IL17RA Binding Assay is designed to measure the interaction between IL17A and IL17RA. 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 IL17A and IL17RA 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 IL17A and IL17RA 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 IL17A/IL17RA interaction. Thus, compound or antibody blocking IL17A/IL17RA interaction will cause a reduction in HTRF signal.

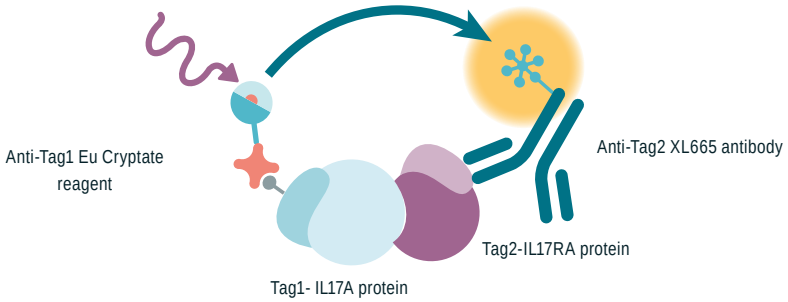
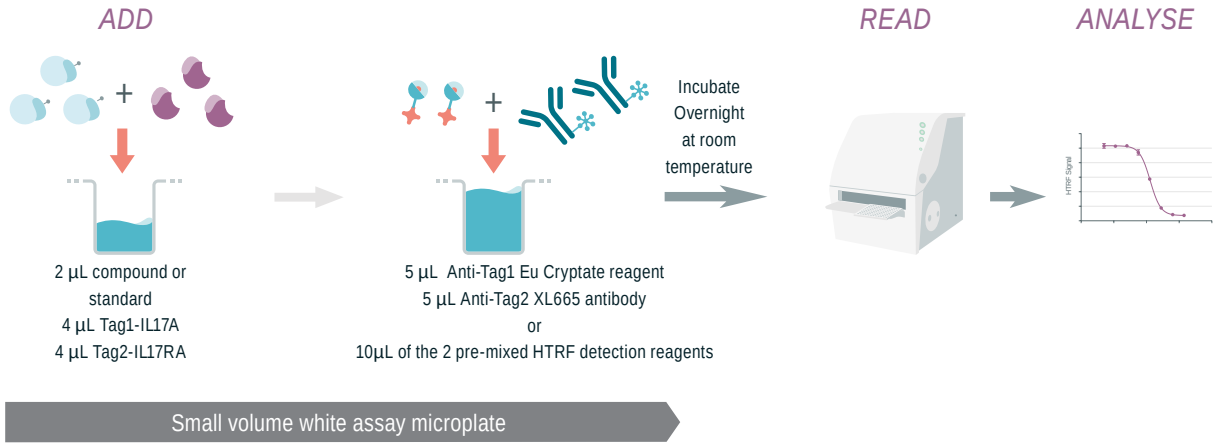



Figure 1: Principle of the HTRF IL17A/IL17RA assay.

## MANUAL AT A GLANCE



## MATERIALS:

| KIT COMPONENTS | 500 TESTS<br>CAT # 64BDIL17PEG | 10,000 TESTS<br>CAT # 64BDIL17PEH |
|---|---|---|
| Tag1-IL17A<br>Lyophilized | 1 vial | 2 vials |
| Tag2-IL17RA<br>Lyophilized | 1 vial | 2 vials |
| IL17A-IL17RA standard<br>Frozen | 1 vial - 40 µL<br>2.5 µM | 1 vial - 40 µL<br>2.5 µM |
| Anti-Tag1 Eu Cryptate reagent-<br>Frozen | 1 vial - 50 µL<br>50X | 1 vial - 1 mL<br>50X |
| Anti-Tag2 XL665 antibody<br>Frozen | 1 vial - 50 µL<br>50X | 1 vial - 1 mL<br>50X |
| PPI Europium Detection Buffer<br>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 ≤-60°C. Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.

Once reconstituted, tagged IL17A & IL17RA 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 ≤-60°C.

Thawed PPI Europium Detection Buffer can be stored at 2-8°C on your premises.

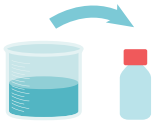
## REAGENT PREPARATION

### 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 homogeneize 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.

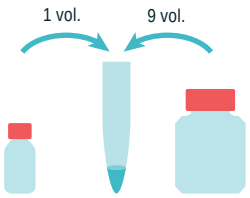
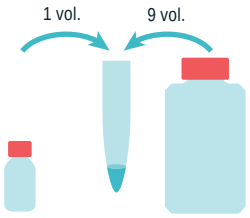
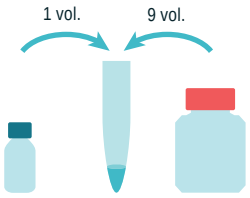
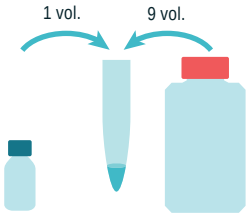
## TO PREPARE STOCK SOLUTIONS:

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-IL17A | | | |
| Reconstitute the Tag1-IL17A with 200 µL of distilled water in order to obtain a 10X stock solution.<br>Mix gently, DO NOT vortex !<br>This stock solution can be frozen and stored at ≤ 60°C. |  |  | Reconstitute the Tag1-IL17A with 2 mL of distilled water in order to obtain a 10X stock solution.<br>Mix gently, DO NOT vortex !<br>This stock solution can be frozen and stored at ≤ 60°C. |
| Tag2-IL17RA | | | |
| Reconstitute the Tag2-IL17RA with 200 µL of distilled water in order to obtain a 10X stock solution.<br>Mix gently, DO NOT vortex !<br>This stock solution can be frozen and stored at ≤ 60°C. |  |  | Reconstitute the Tag2-IL17RA with 2 mL of distilled water in order to obtain a 10X stock solution.<br>Mix gently, DO NOT vortex !<br>This stock solution can be frozen and stored at ≤ 60°C. |
| IL17A-IL17RA Standard | | | |
| Thaw the IL17A-IL17RA standard.<br>Mix gently.<br>This 5X standard stock solution can be frozen and stored at ≤ 60°C. |  |  | Thaw the IL17A-IL17RA standard.<br>Mix gently.<br>This 5X standard stock solution can be frozen and stored at ≤ 60°C. |
| Anti-Tag1 Eu Cryptate reagent | | | |
| Thaw the Anti-Tag1 Eu Cryptate reagent.<br>Mix gently.<br>This 50X Anti-Tag1 Eu Cryptate reagent stock solution can be frozen and stored at ≤ 60°C. |  |  | Thaw the Anti-Tag1 Eu Cryptate reagent.<br>Mix gently.<br>This 50X Anti-Tag1 Eu Cryptate reagent stock solution can be frozen and stored at ≤ 60°C. |
| Anti-Tag2 XL665 antibody | | | |
| Thaw the Anti-Tag2 XL665 antibody.<br>Mix gently.<br>This 50X Anti-Tag2 XL665 antibody stock solution can be frozen and stored at ≤ 60°C. |  |  | Thaw the Anti-Tag2 XL665 antibody.<br>Mix gently.<br>This 50X Anti-Tag2 XL665 antibody stock solution.can be frozen and stored at ≤ 60°C. |
| PPI Europium Detection Buffer | | | |
| Thaw the PPI Europium Detection Buffer<br>The thawed buffer can be stored at 2-8°C on your premises. |  |  | Thaw the PPI Europium Detection Buffer<br>The thawed buffer can be stored at 2-8°C on your premises.. |

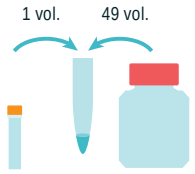
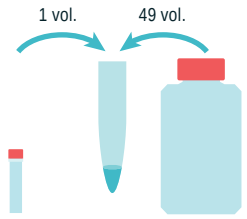
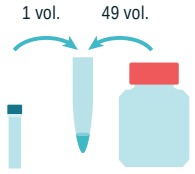
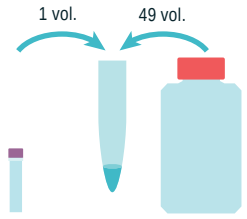
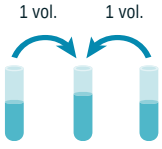
## TO PREPARE TAG1-IL17A AND TAG2-IL17RA WORKING SOLUTIONS:

Each well requires 4 µL of each Tag-protein.

| 500 TESTS | | 10,000 TESTS | |
|---|---|---|---|
| Tag1-IL17A | | | |
| Dilute 10-fold the 10X stock solution (reconstituted reagent) of Tag1-IL17A with PPI Europium Detection Buffer:<br>e.g. 200 µL of reconstituted Tag1-IL17A stock solution + 1800 µL of PPI Europium Detection Buffer. |  |  | Dilute 10-fold the 10X stock solution (reconstituted reagent) of Tag1-IL17A with PPI Europium Detection Buffer:<br>e.g. 2 mL of reconstituted Tag1-IL17A stock solution + 18 mL of PPI Europium Detection Buffer. |
| Tag2-IL17RA | | | |
| Dilute 10-fold the 10X stock solution (reconstituted reagent) of Tag2-IL17RA with PPI Europium Detection Buffer :<br>e.g. 200 µL of reconstituted Tag2-IL17RA stock solution + 1800 µL of PPI Europium Detection Buffer. |  |  | Dilute 10-fold the 10X stock solution (reconstituted reagent) of Tag2-IL17RA with PPI Europium Detection Buffer :<br>e.g. 2 mL of reconstituted Tag2-IL17RA stock solution + 18 mL of PPI Europium Detection Buffer. |

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

Each well requires 5 µL of each anti-Tag donor & acceptor reagents.

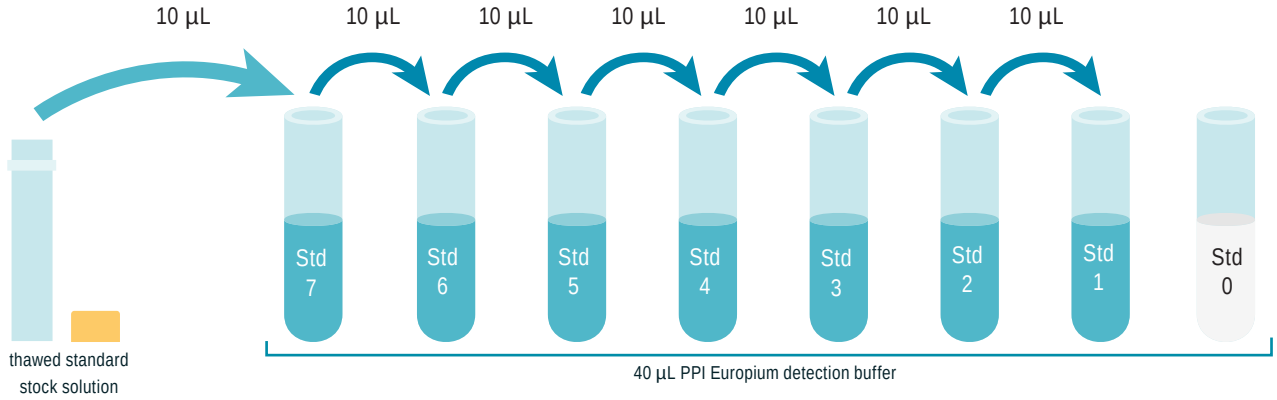
| 500 TESTS | | 10,000 TESTS | |
|---|---|---|---|
| Anti-Tag1 Eu Cryptate reagent | | | |
| Dilute 50-fold the 50X stock solution (thawed reagent) of Anti-Tag1 Eu Cryptate reagent with PPI Europium Detection Buffer :<br>e.g. 50 µL of reconstituted Anti-Tag1 Eu Cryptate reagent stock solution + 2450 µL of PPI Europium Detection Buffer . |  |  | Dilute 50-fold the 50X stock solution (thawed reagent) of Anti-Tag1 Eu Cryptate reagent with PPI Europium Detection Buffer :<br>e.g. 1 mL of reconstituted Anti-Tag1 Eu Cryptate reagent 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 :<br>e.g. 50 µL of reconstituted Anti-Tag2 XL665 antibody stock solution + 2450 µL of PPI Europium Detection Buffer . |  |  | Dilute 50-fold the 50X stock solution (thawed reagent) of Anti-Tag2 XL665 antibody with PPI Europium Detection Buffer :<br>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:<br>e.g. 2.5 mL of Anti-Tag1 Eu Cryptate reagent + 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:<br>e.g. 20 mL of Anti-Tag1 Eu Cryptate reagent + 20 mL of Anti-Tag2 XL665 antibody |

## TO PREPARE WORKING IL17A-IL17RA STANDARD SOLUTIONS:

- Each well requires 2 µL 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 IL17A-IL17RA standard stock solution = 2.5 µM (2 500 000 pM)
2. Prepare the following dilutions:
   •Dilute the thawed standard stock solution 5-fold with PPI Europium detection buffer.
   In practice: take 10 µL of stock solution and add it to 40 µL of PPI Europium detection buffer. Mix gently. This yields the high standard (Std 7: 500 000 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 µL of PPI Europium detection buffer into each vial from Std 6 to Std 0
   - Add 10 µL of standard to 40 µL 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 IL17A-IL17RA standard stock solution | 2.5 µM (2 500 000 pM) | |
| Standard 7 | 10 µL standard stock solution + 40 µL PPI Europium detection buffer | 500 000 pM | 50 000 pM |
| Standard 6 | 10 µL Standard 7 + 40 µL PPI Europium detection buffer | 100 000 pM | 10 000 pM |
| Standard 5 | 10 µL Standard 6 + 40 µL PPI Europium detection buffer | 20 000 pM | 2 000 pM |
| Standard 4 | 10 µL Standard 5 + 40 µL PPI Europium detection buffer | 4 000 pM | 400 pM |
| Standard 3 | 10 µL Standard 4 + 40 µL PPI Europium detection buffer | 800 pM | 80 pM |
| Standard 2 | 10 µL Standard 3 + 40 µL PPI Europium detection buffer | 160 pM | 16 pM |
| Standard 1 | 10 µL Standard 2 + 40 µL PPI Europium detection buffer | 32 pM | 3.2 pM |
| Standard 0 | 40 µL PPI Europium detection buffer | 0 pM | 0 pM |

## ASSAY MANUAL

| | Standard | Samples |
|---|---|---|
| STEP 1  | Dispense into each standard well<br>2 µL of standard<br>4 µL of Tag1-IL17A<br>4 µL of Tag2-IL17RA. | Dispense into each sample well<br>2 µL of compound/antibody or buffer<br>4 µL of Tag1-IL17A<br>4 µL of Tag2-IL17RA. |
| STEP 2  | Dispense into all standard & sample wells 10 µL of pre-mixed<br>Anti-Tag1 Eu Cryptate reagent and Anti-Tag2 XL665 antibody | |
| STEP 3  | Seal the plate and incubate for Overnight.at room temperature | |
| STEP 4  | Remove the plate sealer and read on an HTRF® compatible reader. | |

### STANDARD MANUAL FOR INHIBITORY ASSAY IN 20 µL FINAL VOLUME

| | Standard | Inhibitor | Tag1-IL17A | Tag2-IL17RA | Anti-Tag1 Eu Cryptate reagent | Anti-Tag2 XL665 antibody | PPI Europium detection buffer |
|---|---|---|---|---|---|---|---|
| Standard | 2 µL | - | 4 µL | 4 µL | 5 µL | 5 µL | - |
| Sample | - | 2 µL | 4 µL | 4 µL | 5 µL | 5 µL | - |
| Positive control | - | - | 4 µL | 4 µL | 5 µL | 5 µL | 2 µL |
| Negative control | - | - | 4 µL | - | 5 µL | 5 µL | 6 µL |
| Buffer control | - | - | - | - | - | - | 20 µL |

## EXAMPLE OF PLATE MAP

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A | Buffer control:<br>20 µL PPI Europium detection buffer | Repeat Well A1 | Repeat Well A1 | Compound...:<br>2 µL compound...<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well A4 | Repeat Well A4 |
| B | Negative control:<br>6 µL PPI Europium detection buffer<br>4 µL Tag1-IL17A<br>10 µL pre-mix anti-Tag reagents | Repeat Well B1 | Repeat Well B1 | Compound...:<br>2 µL compound...<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well B4 | Repeat Well B4 |
| C | Positive control:<br>2 µL PPI Europium detection buffer<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well C1 | Repeat Well C1 | Compound...:<br>2 µL compound...<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well C4 | Repeat Well C4 |
| D | Std 0:<br>2 µL Standard 0<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well D1 | Repeat Well D1 | Compound...:<br>2 µL compound...<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well D4 | Repeat Well D4 |
| E | Std 1:<br>2 µL Standard 1<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well E1 | Repeat Well E1 | Compound...:<br>2 µL compound...<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well E4 | Repeat Well E4 |
| F | Std 2:<br>2 µL Standard 2<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well F1 | Repeat Well F1 | Compound...:<br>2 µL compound...<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well F4 | Repeat Well F4 |
| G | Std 3:<br>2 µL Standard 3<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well G1 | Repeat Well G1 | Compound...:<br>2 µL compound...<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well G4 | Repeat Well G4 |
| H | Std 4:<br>2 µL Standard 4<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well H1 | Repeat Well H1 | | | |
| I | Std 5:<br>2 µL Standard 5<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well I1 | Repeat Well I1 | | | |
| J | Std 6:<br>2 µL Standard 6<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>10 µL pre-mix anti-Tag reagents | Repeat Well J1 | Repeat Well J1 | | | |
| K | Std 7:<br>2 µL Standard 7<br>4 µL Tag1-IL17A<br>4 µL Tag2-IL17RA<br>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.

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

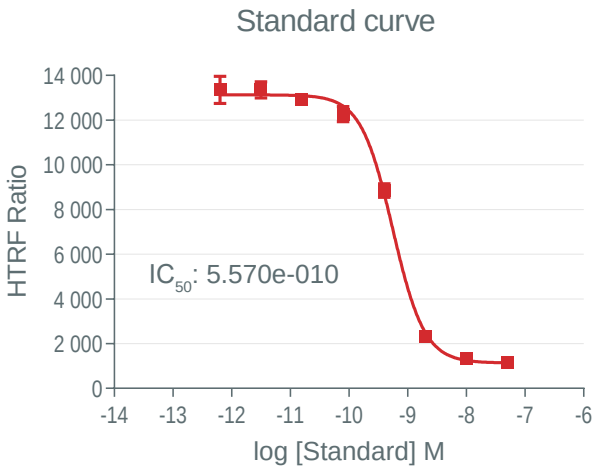
For more information about data reduction, please visit www.revvity.com

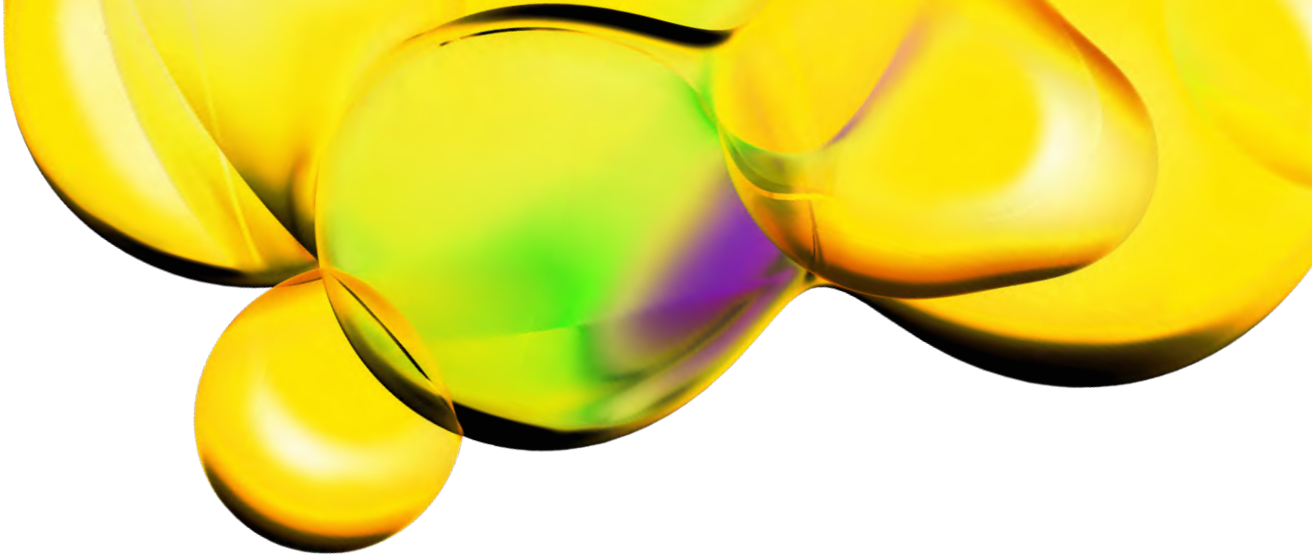
## RESULTS

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.







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