MANUAL

CD47 / SIRP ALPHA BINDING KITS

Part # 64SIRPPEG & 64SIRPPEH

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

Revision: #03 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 CD47 / SIRP alpha Binding Assay is designed to measure the interaction between CD47 and SIRP alpha. 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 CD47 and SIRP alpha 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 CD47 and SIRP alpha 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 CD47 / SIRP alpha interaction. Thus, compound or antibody blocking CD47 / SIRP alpha interaction will cause a reduction in HTRF signal.

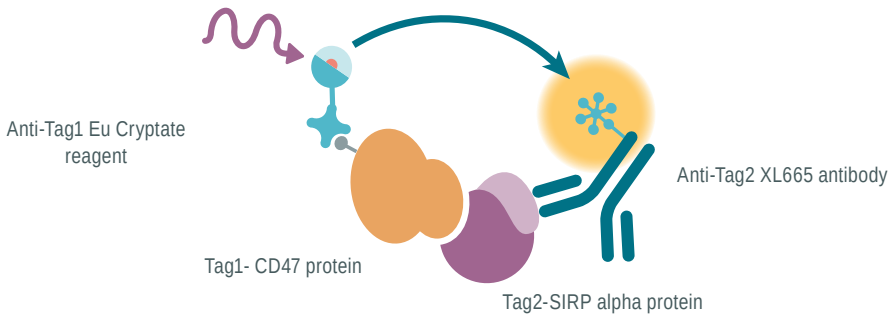
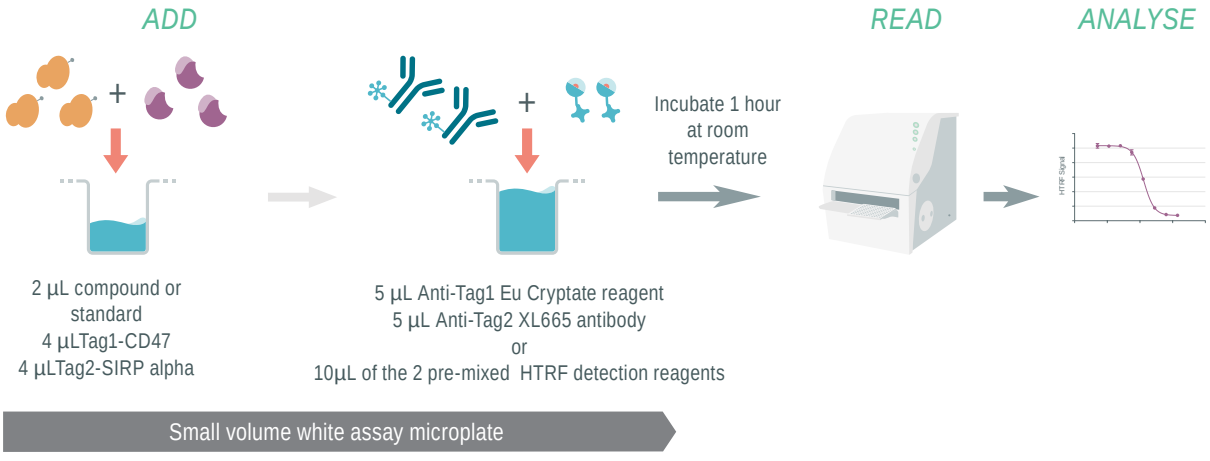


Figure 1: Principle of the HTRF CD47 / SIRP alpha assay.

MANUAL AT A GLANCE



MATERIALS:

KIT COMPONENTS	500 TESTS CAT # 64SIRPPEG	10,000 TESTS CAT # 64SIRPPEH
Tag1-CD47 Frozen	1 vial - 50 µL 40X	1 vial - 1 mL 40X
Tag2-SIRP alpha Frozen	1 vial - 50 µL 40X	1 vial - 1 mL 40X
CD47 / SIRP alpha standard Frozen	1 vial - 50 µL 2.5 µM	1 vial - 50 µL 2.5 µM
Anti-Tag1 Eu Cryptate reagent Frozen	1 vial - 50 µL 50X	1 vial - 1 mL 50X
Anti-Tag2 XL665 antibody Frozen	1 vial - 50 µL 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.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 CD47 & SIRP alpha 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 buffer 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. We recommend keeping DMSO below 1% during the assay (20 µL final volume).

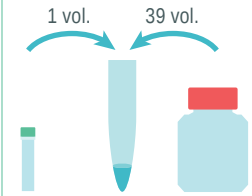
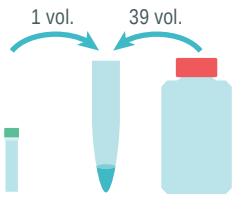
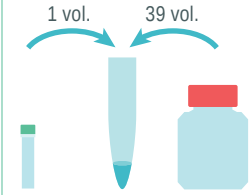
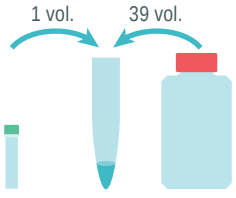
TO PREPARE STOCK SOLUTIONS:

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-CD47			
Thaw the Tag1-CD47. Mix gently. This 40X stock solution can be frozen and stored at ≤ -60°C.			Thaw the the Tag1-CD47. Mix gently This 40X stock solution can be frozen and stored at ≤ -60°C.
Tag2-SIRP alpha			
Thaw the Tag2-SIRP alpha. Mix gently. This 40X stock solution can be frozen and stored at ≤ -60°C.			Thaw the Tag2-SIRP alpha. Mix gently. This 40X stock solution can be frozen and stored at ≤ -60°C.
CD47 / SIRP alpha Standard			
Thaw the CD47 / SIRP alpha standard. Mix gently. This standard stock solution can be frozen and stored at ≤ -60°C.			Thaw the CD47 / SIRP alpha standard. Mix gently. This standard stock solution can be frozen and stored at ≤ -60°C.
Anti-Tag1 Eu Cryptate reagent			
Thaw the Anti-Tag1 Eu Cryptate reagent. Mix gently. This 50X Eu stock solution can be frozen and stored at ≤ -60°C.			Thaw the Anti-Tag1 Eu Cryptate reagent. Mix gently. This 50X Eu stock solution can be frozen and stored at ≤ -60°C.
Anti-Tag2 XL665 antibody			
Thaw the Anti-Tag2 XL665 antibody. Mix gently. This 50X XL665 stock solution can be frozen and stored at ≤ -60°C.			Thaw the Anti-Tag2 XL665 antibody. Mix gently. This 50X XL665 stock solution.can be frozen and stored at ≤ -60°C.
PPI Europium Detection Buffer			
Thaw the PPI Europium Detection Buffer The thawed buffer can be stored at 2-8°C on your premises.			Thaw the PPI Europium Detection Buffer The thawed buffer can be stored at 2-8°C on your premises.

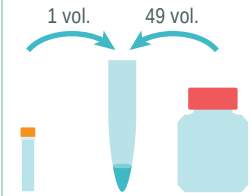
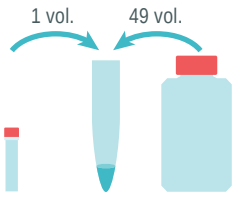
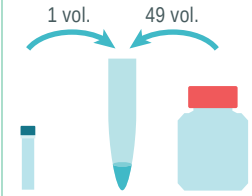
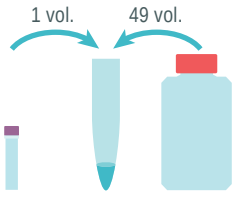
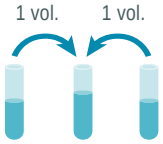
TO PREPARE TAG1-CD47 AND TAG2-SIRP ALPHA WORKING SOLUTIONS:

Each well requires 4 µL of each Tag-protein.

500 TESTS		10,000 TESTS	
Tag1-CD47			
Dilute 40-fold the stock solution (thawed reagent) of Tag1-CD47 with PPI Europium Detection Buffer: e.g. 50 µL of reconstituted Tag1-CD47 stock solution + 1950 µL of PPI Europium Detection Buffer.			Dilute 40-fold the stock solution (reconstituted reagent) of Tag1-CD47 with PPI Europium Detection Buffer: e.g. 1 mL of reconstituted Tag1-CD47 stock solution + 39 mL of PPI Europium Detection Buffer.
Tag2-SIRP alpha			
Dilute 40-fold the stock solution (reconstituted reagent) of Tag2-SIRP alpha with PPI Europium Detection Buffer: e.g. 50 µL of reconstituted Tag2-SIRP alpha stock solution + 1950 µL of PPI Europium Detection Buffer.			Dilute 40-fold the stock solution (reconstituted reagent) of Tag2-SIRP alpha with PPI Europium Detection Buffer: e.g. 1 mL of reconstituted Tag2-SIRP alpha stock solution + 39 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: 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: 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: 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: 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 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: e.g. 20 mL of Anti-Tag1 Eu Cryptate reagent + 20 mL of Anti-Tag2 XL665 antibody

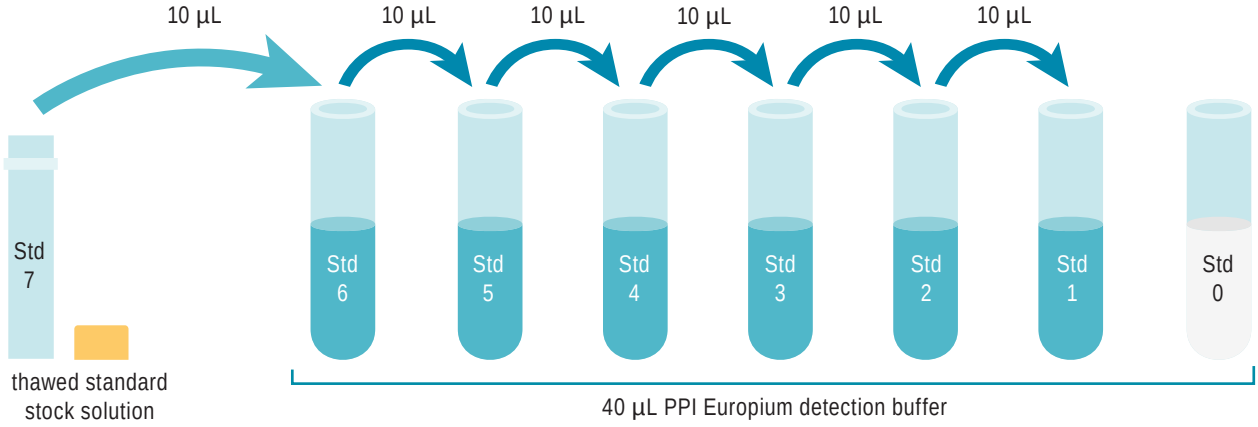
TO PREPARE WORKING CD47 / SIRP ALPHA STANDARD SOLUTIONS:

- 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:

- Thaw the CD47 / SIRP alpha standard stock solution, this yields the high standard (Std 7: 2.5 µM (2 500 000 pM)).
- 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 7 Standard Stock solution	Thaw the CD47 / SIRP alpha standard stock solution	2 500 000 pM	250 000 pM
Standard 6	10 µL Standard 7 + 40 µL PPI Europium detection buffer	500 000 pM	50 000 pM
Standard 5	10 µL Standard 6 + 40 µL PPI Europium detection buffer	100 000 pM	10 000 pM
Standard 4	10 µL Standard 5 + 40 µL PPI Europium detection buffer	20 000 pM	2 000 pM
Standard 3	10 µL Standard 4 + 40 µL PPI Europium detection buffer	4 000 pM	400 pM
Standard 2	10 µL Standard 3 + 40 µL PPI Europium detection buffer	800 pM	80 pM
Standard 1	10 µL Standard 2 + 40 µL PPI Europium detection buffer	160 pM	16 pM
Standard 0	40 µL PPI Europium detection buffer	0 pM	0 pM

ASSAY MANUAL

	Standard	Samples
Step 1 	Dispense into each standard well 2 µL of standard 4 µL of Tag1-CD47 4 µL of Tag2-SIRP alpha.	Dispense into each sample well 2 µL of compound/antibody or buffer 4 µL of Tag1-CD47 4 µL of Tag2-SIRP alpha.
Step 2 	Dispense into all standard & sample wells 10 µL of pre-mixed Anti-Tag1 Eu Cryptate reagent and Anti-Tag2 XL665 antibody	
Step 3 	Seal the plate and incubate for 1 hour.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-CD47	Tag2-SIRP alpha	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: 20 µL PPI Europium detection buffer	Repeat Well A1	Repeat Well A1	Compound...: 2 µL compound... 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 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-CD47 10 µL pre-mix anti-Tag reagents	Repeat Well B1	Repeat Well B1	Compound...: 2 µL compound... 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 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-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well C1	Repeat Well C1	Compound...: 2 µL compound... 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well C4	Repeat Well C4
D	Std 0: 2 µL Standard 0 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well D1	Repeat Well D1	Compound...: 2 µL compound... 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well D4	Repeat Well D4
E	Std 1: 2 µL Standard 1 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well E1	Repeat Well E1	Compound...: 2 µL compound... 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well E4	Repeat Well E4
F	Std 2: 2 µL Standard 2 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well F1	Repeat Well F1	Compound...: 2 µL compound... 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well F4	Repeat Well F4
G	Std 3: 2 µL Standard 3 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well G1	Repeat Well G1	Compound...: 2 µL compound... 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well G4	Repeat Well G4
H	Std 4: 2 µL Standard 4 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well H1	Repeat Well H1			
I	Std 5: 2 µL Standard 5 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well I1	Repeat Well I1			
J	Std 6: 2 µL Standard 6 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 10 µL pre-mix anti-Tag reagents	Repeat Well J1	Repeat Well J1			
K	Std 7: 2 µL Standard 7 4 µL Tag1-CD47 4 µL Tag2-SIRP alpha 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.

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

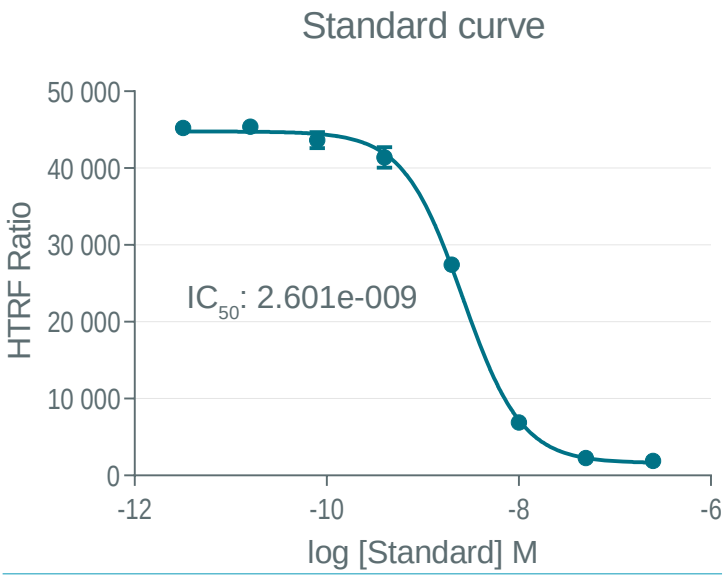
For more information about data reduction, please visit www.revvity.com

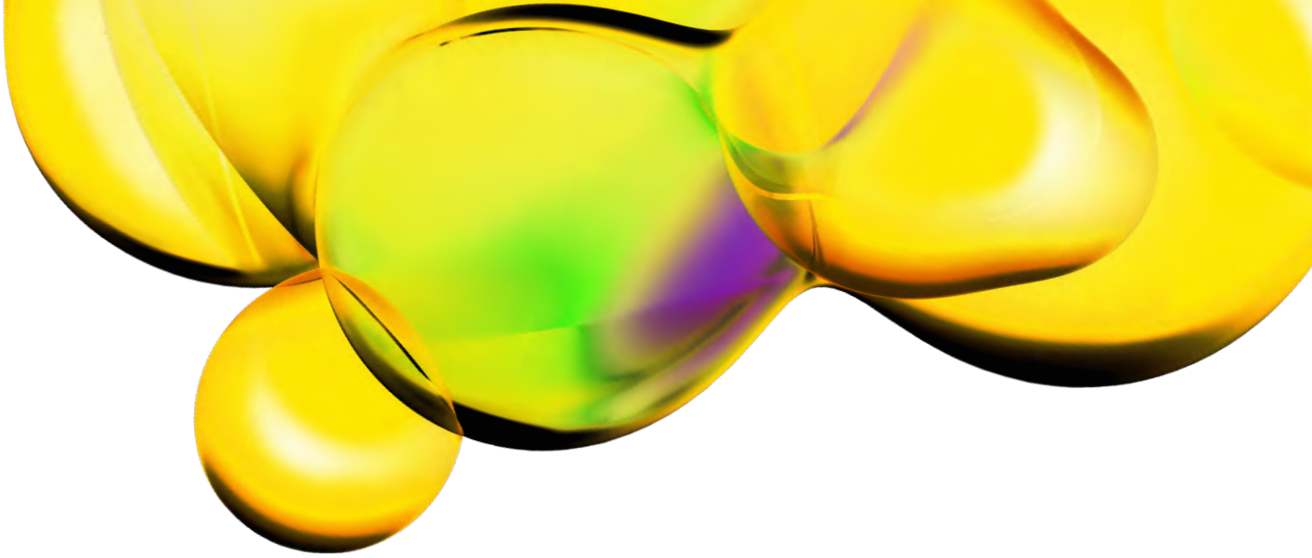
RESULTS

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 an HTRF compatible reader with a flash lamp.

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





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

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