MANUAL

RIG-I BINDING KITS

Part # 64BDRIGIPEG & 64BDRIGIPEH

Test Size#: 500 TESTS (64BDRIGIPEG), 10,000 TESTS (64BDRIGIPEH) - assay volume: 20 µL

Revision: #02 of September 2023 **Store at:** ≤-60°C

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

ASSAY PRINCIPLE

Revvity RIG-I binding assay is only intended for identification and characterization of human RIG-I binders using HTRF® technology.

RIG-I binders are detected in a competitive assay format using a specific 6His antibody labeled with Terbium Cryptate (donor) which binds to Human RIG-I 6His-tagged and HTRF acceptor-streptavidin which binds to biotinylated 3p-dsRNA. The detection principle is based on HTRF® technology. When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). Your compound competes with the couple acceptor-streptavidin/biot-3p-dsRNA, and thereby prevents FRET from occurring. The specific signal is inversely proportional to the compound concentration (Fig. 1).

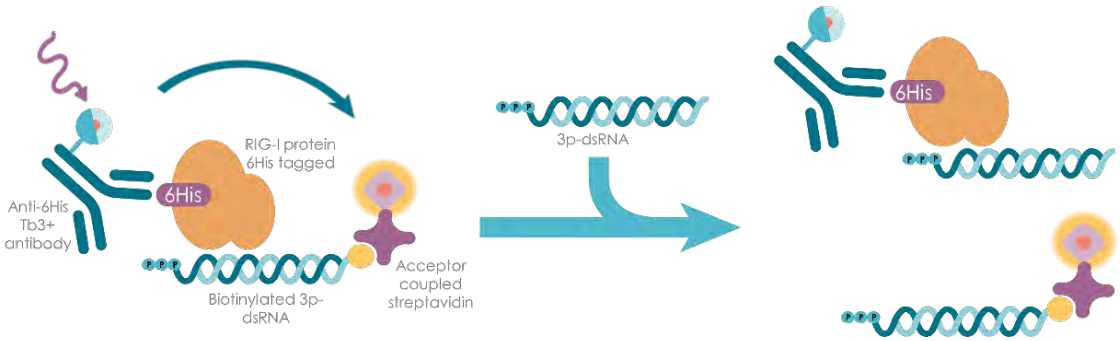
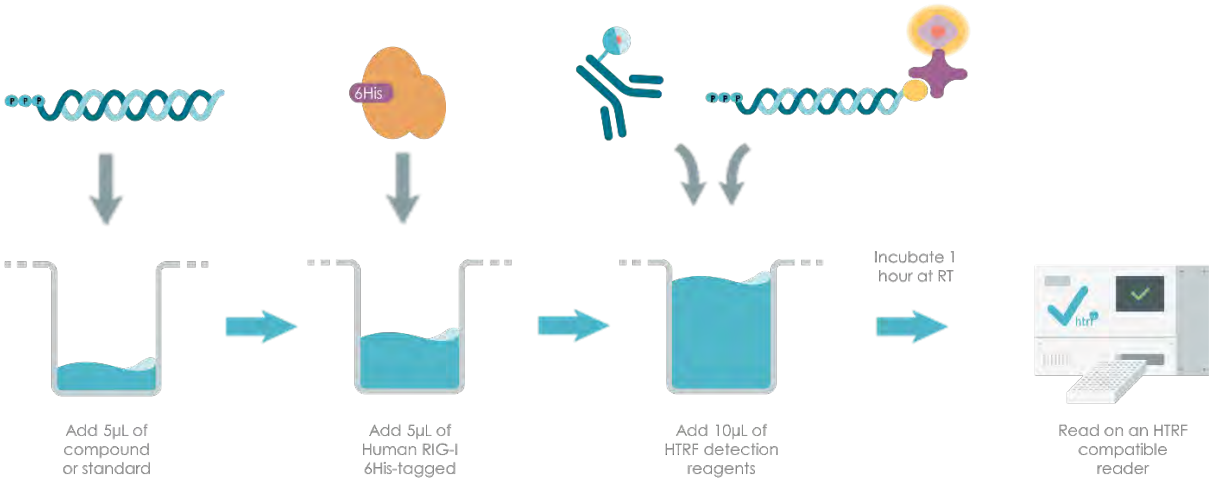


Figure 1: Principle of HTRF® RIG-I binding competitive assay.

MANUAL AT A GLANCE



Make sure you use the appropriate setup for Tb 3+ Cryptate. For more information about setup and HTRF® compatible readers, please visit our website at: www.revvity.com

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS* CAT # 64BDRIGIPEG	10,000 TESTS* CAT # 64BDRIGIPEH
RIG-I standard Frozen	1 vial - 150 µL	2 vials - 150 µL
6His Tb Cryptate Antibody	1 vial - 50 µL	1 vial - 1 mL
Streptavidin-d2	1 vial - 25 µL	1 vial – 500 µL
Biotinylated-3p-dsRNA	1 vial - 25 µL	1 vial – 500 µL
Human RIGI-I 6His-tagged	1 vial - 50 µL	2 vials – 1 mL
Diluent #13	1 bottle – 8mL	1 bottle – 200 mL
Detection buffer #16	4 vials - 2 mL	1 bottle - 200 mL

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 and 10,000 tests respectively in 20 µL final.
Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.

PURCHASE SEPARATELY:

- Low volume white (only) microplate*
- HTRF®-Certified Reader **. Make sure the setup for Tb3+ Cryptate is used.

* For HTRF microplate recommendations, please visit www.revvity.com
** For a list of HTRF-compatible readers and setup recommendations, please visit www.revvity.com

STORAGE AND STABILITY

Store the kit at -60°C or below. Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.

Thaw and aliquot the protein on ice.

Once thawed, other 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 or below.

Volume of reagent aliquots should not be under 10 µL.

Thawed diluent and 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 buffers.
- Thaw protein on ice, other reagents can be thawed at room temperature
- Before use, allow buffer to warm up at room temperature and homogenize it with a vortex.

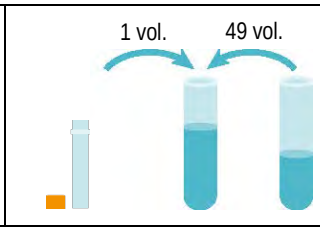
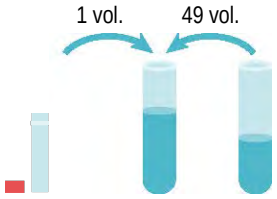
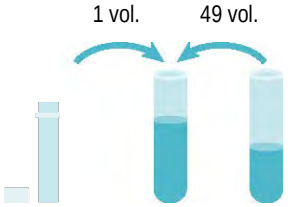
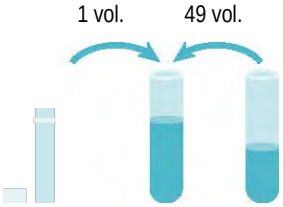
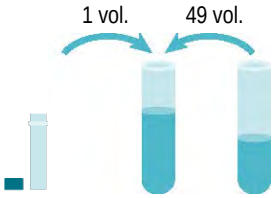
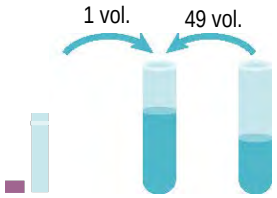
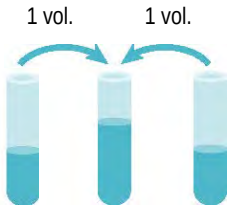
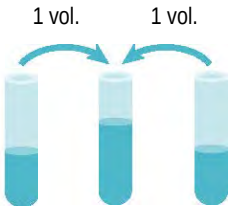
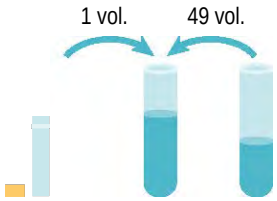
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64BDRIGIPEG		10,000 TESTS KIT - 64BDRIGIPEH	
6His Cryptate antibody			
Thaw the 6His Tb Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.			Thaw the 6His Tb Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.
Acceptor detection reagents			
Thaw the biotinytated-3p-dsRNA. Centrifuge. This 100X stock solution can be frozen and stored at -60°C or below.			Thaw the biotinytated-3p-dsRNA. Centrifuge. This 100X stock solution can be frozen and stored at -60°C or below.
Thaw the Streptavidin-d2. Centrifuge. This 100X stock solution can be frozen and stored at -16°C or below.			Thaw the Streptavidin-d2. Centrifuge. This 100X stock solution can be frozen and stored at -16°C or below.
Human RIG-I 6His-tagged			
Thaw the Human RIG-I 6His-tagged on ice. Centrifuge the vial. This solution can be frozen and stored at -60°C or below. Freeze / Thaw cycle are not recommended.			Thaw the Human RIG-I 6His-tagged on ice. Centrifuge the vial. This solution can be frozen and stored at -60°C or below. Freeze / Thaw cycle are not recommended.
RIG-I binding standard			
Thaw the RIG-I binding standard. Centrifuge. This solution can be frozen and stored at -60°C or below.			Thaw the RIG-I binding standard. Centrifuge. This solution can be frozen and stored at -60°C or below.
Buffers			
Diluent #13 Ready to use Could be stored at 4°C.			Diluent #13 Ready to use Could be stored at 4°C.
Detection buffer #16 Ready to use Could be stored at 4°C.			Detection buffer #16 Ready to use Could be stored at 4°C.

TO PREPARE WORKING SOLUTIONS:

Each well requires 5 µL of each reagent. Prepare in separate vials.

<table>
<tr><th colspan="2">500 TESTS KIT - 64BDRIGIPEG</th><th colspan="2">10,000 TESTS KIT - 64BDRIGIPEH</th></tr>
<tr><td colspan="4">6His Tb Cryptate antibody</td></tr>
<tr><td>Dilute 50-fold the 50X stock solution (thawed reagent) of 6His Tb cryptate antibody with detection buffer, eg 10 µL of thawed Tb cryptate antibody stock solution + 490 µL of detection buffer.</td><td> 
</td><td> 
</td><td>Dilute 50-fold the 50X stock solution (thawed reagent) of 6His Tb cryptate antibody with detection buffer, eg 1mL of thawed Tb cryptate antibody stock solution + 49mL of detection buffer.</td></tr>
<tr><td colspan="4">Acceptor detection mix</td></tr>
<tr><td>Prepare biotinylated-3p-dsRNA 2X: Dilute 50-fold the 100X stock solution (thawed reagent) of biotinylated-3p-dsRNA with detection buffer, eg 5 µL of thawed biotinylated-3p-dsRNA stock solution + 245µL of detection buffer.</td><td> 
</td><td> 
</td><td>Prepare biotinylated-3p-dsRNA 2X: Dilute 50-fold the 100X stock solution (thawed reagent) of biotinylated-3p-dsRNA with detection buffer, eg 50µL of thawed biotinylated-3p-dsRNA stock solution + 2.45mL of detection buffer.</td></tr>
<tr><td>Prepare Streptavidin-d2 2X: Dilute 50-fold the 100X stock solution (thawed reagent) of d2-streptavidin with detection buffer, eg 5 µL of thawed d2-streptavidin stock solution + 245µL of detection buffer.</td><td> 
</td><td> 
</td><td>Prepare Streptavidin-d2 2X: Dilute 50-fold the 100X stock solution (thawed reagent) of d2-streptavidin with detection buffer, eg 50µL of thawed d2-streptavidin stock solution + 2.45mL of detection buffer.</td></tr>
<tr><td>Prepare the acceptor detection mix by mixing 1 volume of biotinylated-3p-dsRNA 2X solution and 1 volume of d2-streptavidin 2X solution, eg 250 µL of biotinylated-3p-dsRNA 2X solution and 250 µL volume of d2-streptavidin 2X solution.</td><td> 
</td><td> 
</td><td>Prepare the acceptor detection mix by mixing 1 volume of biotinylated-3p-dsRNA 2X solution and 1 volume of ds-streptavidin 2x solution, eg 2.5 mL of biotinylated-3p-dsRNA 2X solution and 2.5 mL volume of d2-streptavidin 2X solution.</td></tr>
<tr><td colspan="4">Human RIG-I 6His-tagged</td></tr>
<tr><td>Dilute 50-fold the 50X stock solution of Human RIG-I 6His-tagged protein with detection buffer, eg 10 µL of thawed Human RIG-I 6His-tagged protein stock solution + 490 µL of detection buffer.</td><td colspan="2"> 
</td><td>Dilute 50-fold the 50X stock solution of Human RIG-I 6His-tagged protein with detection buffer, eg 100 µL of thawed Human RIG-I 6His-tagged protein stock solution + 4900 µL of detection buffer.</td></tr>
</table>

TO PREPARE WORKING STANDARD SOLUTIONS:

- Each well requires 5 µL of standard.
- Dilute the standard stock solution serially with Diluent #13**.**
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

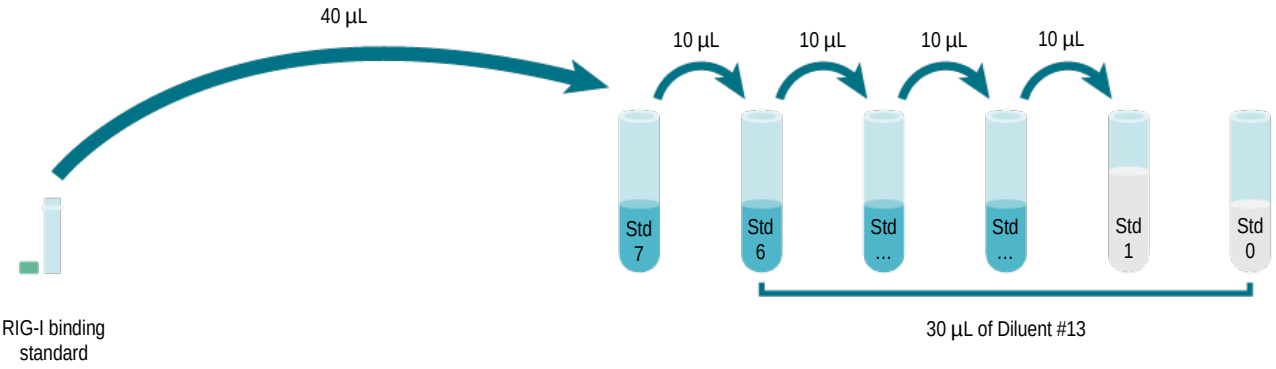
A recommended standard dilution procedure is listed and illustrated below:

The standard stock solution has to be used neat for the high standard (Std 7).

Use the high standard (Std 7) to prepare the standard curve using 1/4 serial dilutions as follows:

- Dispense 30 µL of Diluent #13 into each vial from Std 6 to Std 0.
- Add 10 µL of standard to 30 µL of diluent, mix gently and repeat the 1/4 serial dilution to make standard solutions: Std6, Std5, Std4, Std3, Std2, Std1.

This will create 7 standards for the analyte. Std 0 (Positive control) is diluent alone.



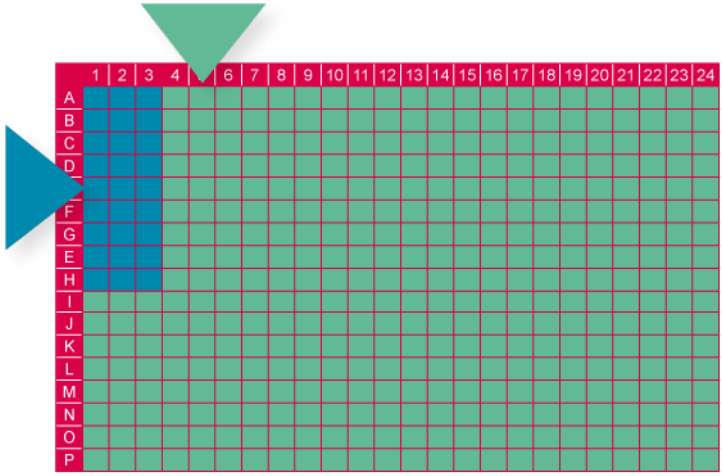
STANDARD	SERIAL DILUTIONS	"RIG-I STANDARD SOLUTION (NM)"
Standard 7	stock solution	1720
Standard 6	10 µL standard 7 + 30 µL diluent #13	430
Standard 5	10 µL standard 6 + 30 µL diluent #13	108
Standard 4	10 µL standard 5 + 30 µL diluent #13	27
Standard 3	10 µL standard 4 + 30 µL diluent #13	6.7
Standard 2	10 µL standard 3 + 30 µL diluent #13	1.7
Standard 1	10 µL standard 2 + 30 µL diluent #13	0.42
Standard 0	40 µL diluent #13	0

ASSAY MANUAL

	NEGATIVE CONTROL (OR CRYPTATE CONTROL)	STANDARD (STD 0 - STD 7)	COMPOUND
Step 1 	Dispense 5 µL of diluent into each negative control well.	Dispense 5 µL of each RIG-I standard (Std 0 - Std 7) into each standard well.	Dispense 5 µL of compound into each compound well.
Step 2 	Add 5 µL of detection buffer to all wells	Add 5 µL of Human RIG-I 6His-tagged protein to all wells	
Step 3 	Add 5 µL of premixed acceptor detection reagent working solution And Add 5µL of 6His Tb antibody working solution to all wells		
Step 4 	Seal the plate and incubate 1 hour at RT		
Step 5 	Remove the plate sealer and read on an HTRF® compatible reader		

EXAMPLE OF PLATE MAP

	1	2	3	4	5	6
A	5 µL diluent (Negative control) 5 µL of detection buffer 10 µL of detection reagents	Repeat Well A1	Repeat Well A1	5 µL Compound 1 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well A4	Repeat Well A4
B	5 µL Std 0 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well B1	Repeat Well B1	5 µL Compound 2 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well B4	Repeat Well B4
C	5 µL Std 1 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well C1	Repeat Well C1	5 µL Compound 3 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well C4	Repeat Well C4
D	5 µL Std ... 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well D1	Repeat Well D1	5 µL Compound ... 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well D4	Repeat Well D4
E	5 µL Std ... 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well E1	Repeat Well E1	5 µL Compound ... 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well E4	Repeat Well E4
F	5 µL Std ... 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well F1	Repeat Well F1	5 µL Compound ... 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well F4	Repeat Well F4
G	5 µL Std ... 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well G1	Repeat Well G1	5 µL Compound ... 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well G4	Repeat Well G4
H	5 µL Std ... 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well H1	Repeat Well H1	5 µL Compound ... 5 µL of Human RIG-I 6His-tagged 10 µL of detection reagents	Repeat Well H4	Repeat Well H4



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.

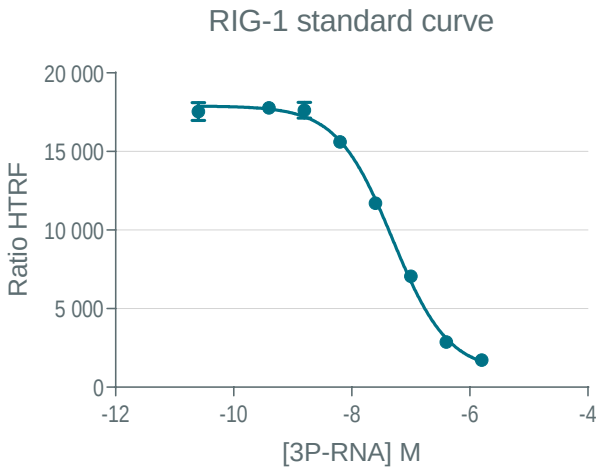
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

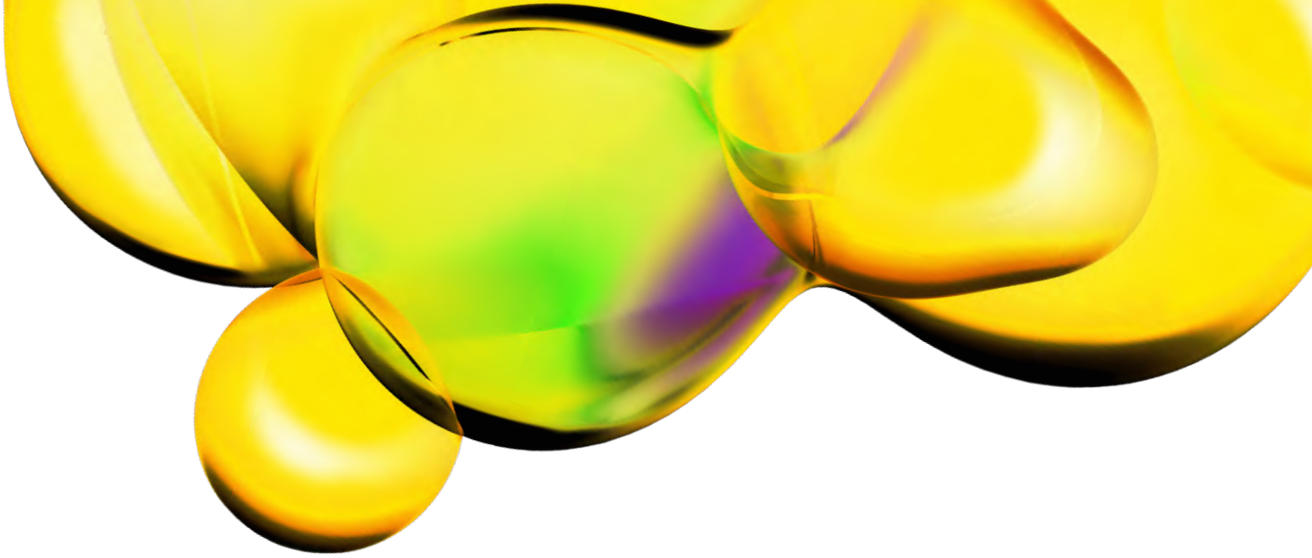
For more information about data reduction, please visit www.revvity.com

RESULTS

This data 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). Results may vary from one HTRF® compatible reader to another.

	RATIO (1)	CV (2)
Std 0	17544	3.2
Std 1 – 0.42 nM	17768	1.6
Std 2 – 1.7 nM	17633	2.9
Std 3 – 6.7 nM	15613	1.8
Std 4 – 27 nM	11715	1.6
Std 5 – 108 nM	7058	2.2
Std 6 – 430 nM	2883	4.1
Std 7 – 1720 nM	1717	0.8





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

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