MANUAL

HTRF KRAS WT GTP BINDING KITS

Part # 64BDKRASWPEG & 64BDKRASWPEH

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

Revision: #03 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 KRAS WT GTP binding assay is only intended for quantitative measurement of GTP competitors using HTRF® technology.

GTP competitors are detected in a competitive assay format using a specific 6His antibody labeled with Europium Cryptate (donor) which binds to Human KRAS WT 6His-tagged and GTP Red labelled with a Red HTRF acceptor (acceptor). 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 GTP Red labelled with a Red HTRF acceptor, and thereby prevents FRET from occurring. The specific signal is inversely proportional to the compound concentration (Fig. 1).

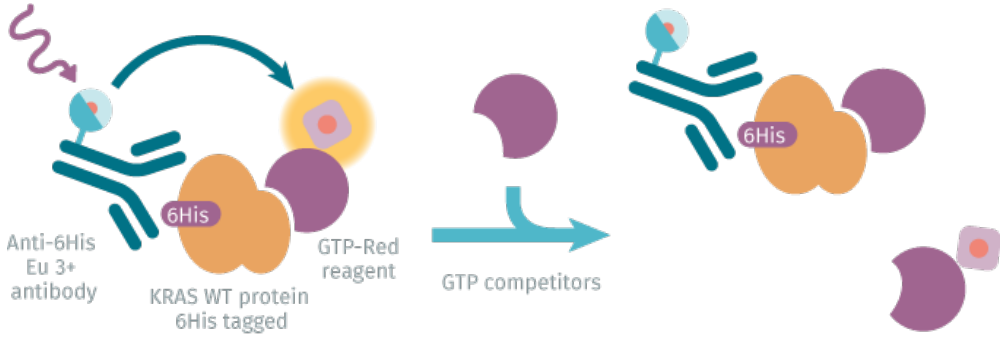
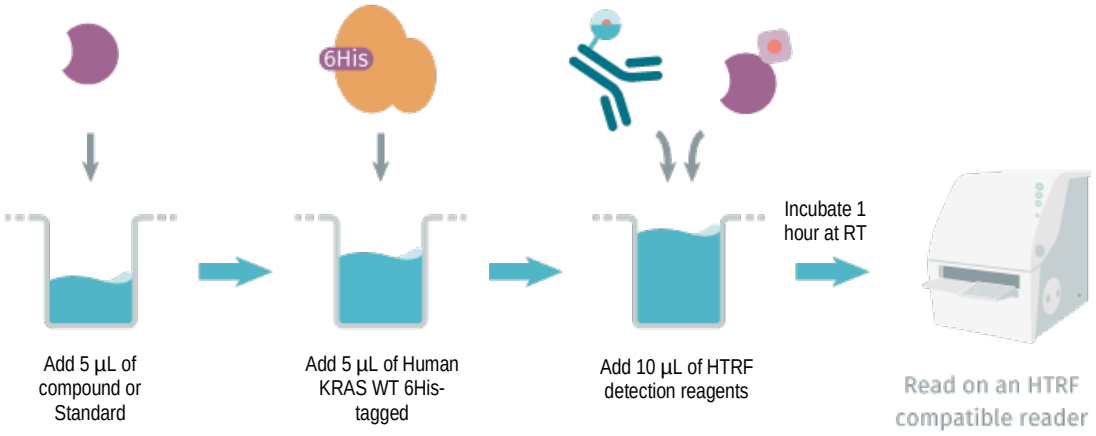


Figure 1: Principle of HTRF® KRAS WT GTP binding competitive assay.

MANUAL AT A GLANCE



Make sure you use the appropriate setup for Eu $^{3+}$ 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 # 64BDKRASWPEG	10,000 TESTS* CAT # 64BDKRASWPEH
KRAS GTP binding standard Frozen - 10X	1 vial - 30 µL	2 vials - 30 µL
6His Eu Cryptate Antibody	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
GTP Red reagent	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
Human KRAS WT 6His-tagged	1 vial - 25 µL Frozen - 100X	2 vials - 250 µL Frozen - 100X
PPI Eu detection buffer ready-to-use	1 vial - 20 mL	1 vial - 220 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 Eu3+ 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 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. The use of an incorrect PPi Eu detection buffer may affect reagent stability and assay results.
- 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.
- KRAS GTP binding standard (for standard curve) must be prepared in PPi Eu detection buffer. The KRAS GTP binding standard is the GDP.

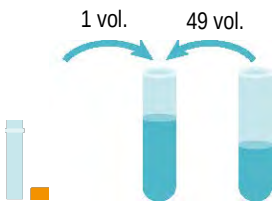
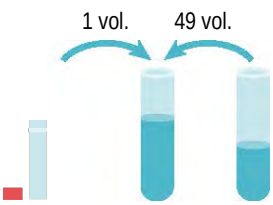
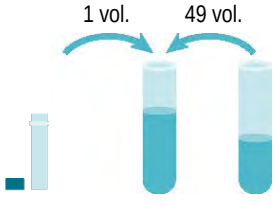
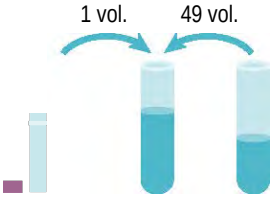
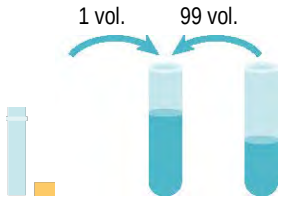
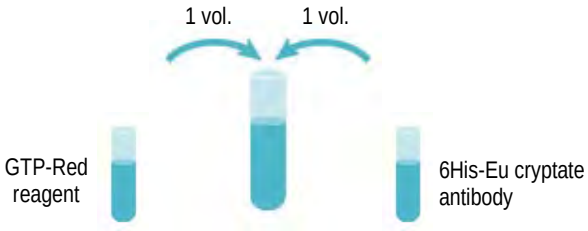
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64BDKRASWPEG		10,000 TESTS KIT - 64BDKRASWPEH	
6His Eu Cryptate antibody			
Thaw the 6His Eu Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.			Thaw the 6His Eu Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.
GTP Red reagent			
Thaw the GTP Red reagent. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.			Thaw the GTP Red reagent. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.
KRAS GTP binding standard			
Thaw the KRAS GTP binding standard. Centrifuge. This 10 X stock solution can be frozen and stored at -16°C or below.			Thaw the KRAS GTP binding standard. Centrifuge. This 10 X stock solution can be frozen and stored at -16°C or below.
Human KRAS WT 6His-tagged			
Thaw the Human KRAS WT 6His-tagged on ice. Centrifuge the vial. To avoid freeze/thaw cycles, it is recommended to aliquot the remainder of this 100X stock solution under 10 µL minimum in disposable plastic vials for storage at ≤-60°C.			Thaw the Human KRAS WT 6His-tagged on ice. Centrifuge the vial. To avoid freeze/thaw cycles, it is recommended to aliquot the remainder of this 100X stock solution under 10 µL minimum in disposable plastic vials for storage at ≤-60°C
PPi Eu detection buffer			
Ready to use.			Ready to use.

TO PREPARE WORKING SOLUTIONS:

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

<table>
<tr><th colspan="2">500 TESTS KIT - 64BDKRASWPEG</th><th colspan="2">10,000 TESTS KIT - 64BDKRASWPEH</th></tr>
<tr><td colspan="4">6His Eu Cryptate antibody</td></tr>
<tr><td>Dilute 50-fold the 50X stock solution (thawed reagent) of 6His Eu cryptate antibody with PPI Eu detection buffer, eg 10 µL of thawed 6His Eu cryptate antibody stock solution + 490 µL of PPI Eu detection buffer.</td><td> 
</td><td> 
</td><td>Dilute 50-fold the 50X stock solution (thawed reagent) of 6His Eu cryptate antibody with PPI Eu detection buffer, eg 1mL of thawed 6His Eu cryptate antibody stock solution + 49mL of PPI Eu detection buffer.</td></tr>
<tr><td colspan="4">GTP Red reagent</td></tr>
<tr><td>Dilute 50-fold the 50X stock solution (thawed reagent) of GTP Red reagent with PPI Eu detection buffer, eg 10 µL of thawed GTP-Red reagent stock solution + 490 µL of PPI Eu detection buffer.</td><td> 
</td><td> 
</td><td>Dilute 50-fold the 50X stock solution (thawed reagent) of GTP Red reagent with PPI Eu detection buffer, eg 1mL of thawed GTP-Red reagent stock solution + 49mL of PPI Eu detection buffer.</td></tr>
<tr><td colspan="4">Human KRAS WT 6His-tagged</td></tr>
<tr><td>Dilute 100-fold the 100X stock solution (thawed reagent on ice) of Human KRAS WT 6His-tagged protein with PPI Eu detection buffer, eg 10 µL of thawed protein stock solution + 990 µL of PPI Eu detection buffer.</td><td colspan="2"> 
</td><td>Dilute 100-fold the 100X stock solution (thawed reagent on ice) of Human KRAS WT 6His-tagged protein with PPI Eu detection buffer, eg 1mL of thawed protein stock solution + 99mL of PPI Eu detection buffer.</td></tr>
<tr><td colspan="4">HTRF reagents</td></tr>
<tr><td>It is possible to pre-mix the two ready-to-use solutions just prior to dispensing the reagents by adding 1 volume of GTP-Red reagent solution to 1 volume of 6His Eu cryptate antibody solution (e.g. 1 mL of GTP-Red reagent + 1 mL of 6His Eu cryptate antibody).</td><td colspan="2"> 
</td><td>It is possible to pre-mix the two ready-to-use solutions just prior to dispensing the reagents by adding 1 volume of GTP-Red reagent solution to 1 volume of 6His Eu cryptate antibody solution (e.g. 1 mL of GTP-Red reagent + 1 mL of 6His Eu cryptate antibody).</td></tr>
</table>

TO PREPARE WORKING STANDARD SOLUTIONS:

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

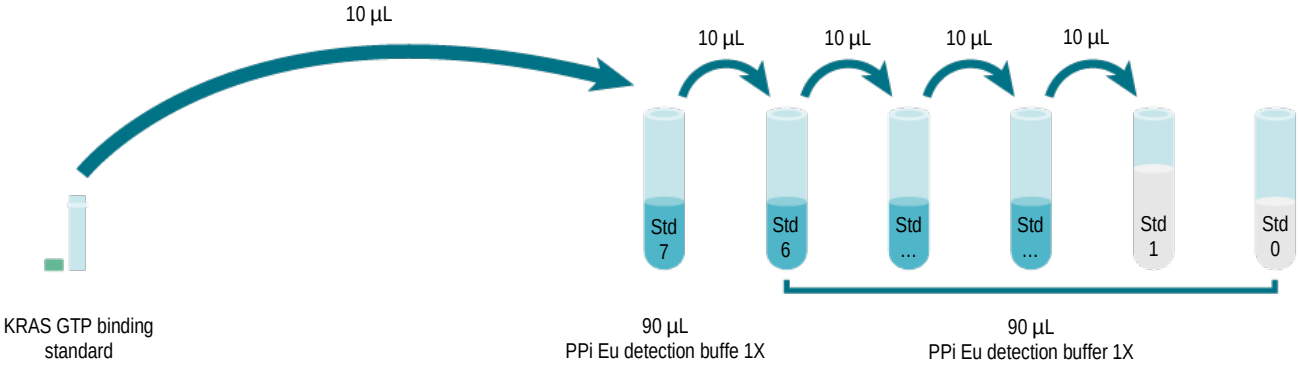
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 10-fold with PPI Eu detection buffer to prepare high standard (Std 7): take 10 µL of standard stock solution and add it to 90 µL of PPI Eu detection buffer. Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/10 serial dilutions as follows:

- Dispense 90 µL of PPi Eu detection buffer into each vial from Std 6 to Std 0.
- Add 10 µL of standard to 90 µL of PPi Eu detection buffer, mix gently and repeat the 1/10 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 PPi Eu detection buffer alone.



STANDARD	SERIAL DILUTIONS	"KRAS GTP BINDING STANDARD WORKING SOLUTION (µM)"	"KRAS GTP BINDING STANDARD FINAL CONCENTRATION (µM)"
Standard Stock solution	Thawed stock solution	4 000	-
Standard 7	10µl standard stock solution + 90 µL PPI Eu detection buffer	400	100
Standard 6	10 µL standard 7 + 90 µL PPI Eu detection buffer	40	10
Standard 5	10 µL standard 6 + 90 µL PPI Eu detection buffer	4	1
Standard 4	10 µL standard 5 + 90 µL PPI Eu detection buffer	0.4	0.1
Standard 3	10 µL standard 4 + 90 µL PPI Eu detection buffer	0.04	0.01
Standard 2	10 µL standard 3 + 90 µL PPI Eu detection buffer	0.004	0.001
Standard 1	10 µL standard 2 + 90 µL PPI Eu detection buffer	0.0004	0.0001
Standard 0	100 µL PPI Eu detection buffer	0	0

TO PREPARE SAMPLES:

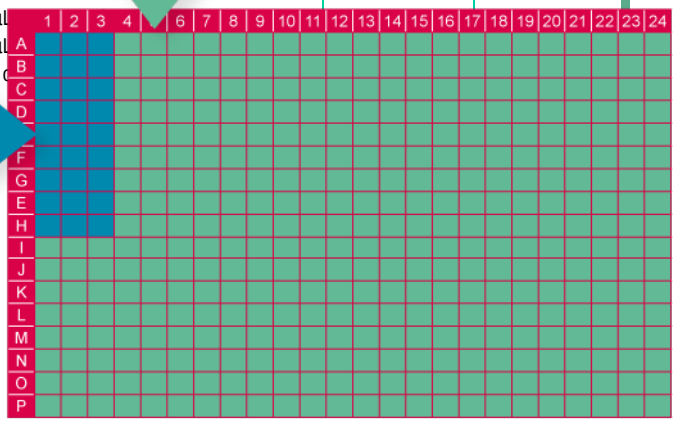
- Each well requires 5 µL of compound.
- Dilute your compound in PPI Eu detection buffer.
- DMSO concentration must not exceed 2% final in the well (fold of change may be impaired by increasing percentage of DMSO).

ASSAY MANUAL

	NEGATIVE CONTROL (OR CRYPTATE CONTROL)	STANDARD (STD 0 - STD 7)	COMPOUND
Step 1 	Dispense 5 µL of PPi Eu detection buffer into each negative control well.	Dispense 5 µL of each KRAS GTP binding standard (Std 0 - Std 7) into each standard well.	Dispense 5 µL of compound into each compound well.
Step 2 	Add 5 µL of PPI Eu detection buffer to all wells	Add 5 µL of Human KRAS WT 6His-tagged protein to all wells	
Step 3 	Add 10 µL of premixed GTP Red reagent and 6His Eu 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 PPi Eu detection buffer (Negative control) A 5 µL of PPI Eu detection buffer 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well A1	Repeat Well A1	5 µL Compound 1 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well A4	Repeat Well A4
B	5 µL Std 0 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well B1	Repeat Well B1	5 µL Compound 2 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well B4	Repeat Well B4
C	5 µL Std 1 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well C1	Repeat Well C1	5 µL Compound 3 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well C4	Repeat Well C4
D	5 µL Std ... 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well D1	Repeat Well D1	5 µL Compound ... 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well D4	Repeat Well D4
E	5 µL Std ... 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well E1	Repeat Well E1	5 µL Compound ... 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well E4	Repeat Well E4
F	5 µL Std ... 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well F1	Repeat Well F1	5 µL Compound ... 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well F4	Repeat Well F4
G	5 µL Std ... 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well G1	Repeat Well G1	5 µL Compound ... 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well G4	Repeat Well G4
H	5 µL Std ... 5 µL of Human KRAS WT 6His-tagged 10 µL of GTP Red- and 6His-Eu premixed	Repeat Well H1	Repeat Well H1	5 µL 5 µL µL		



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.

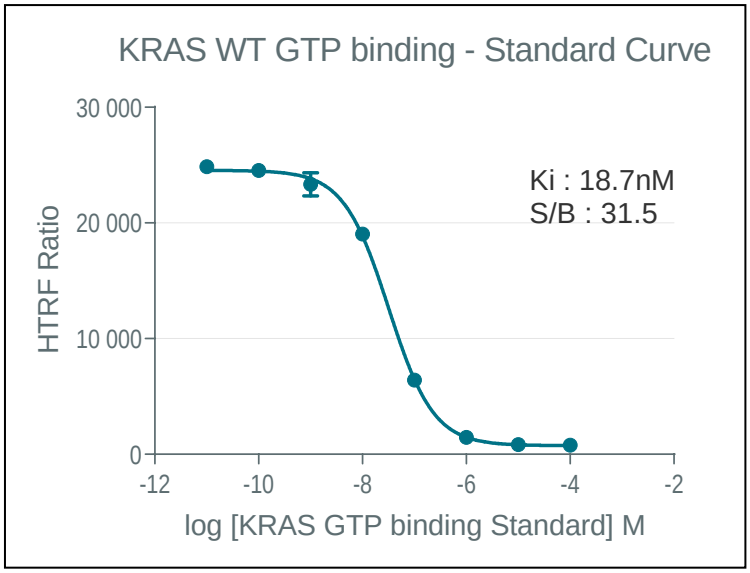
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

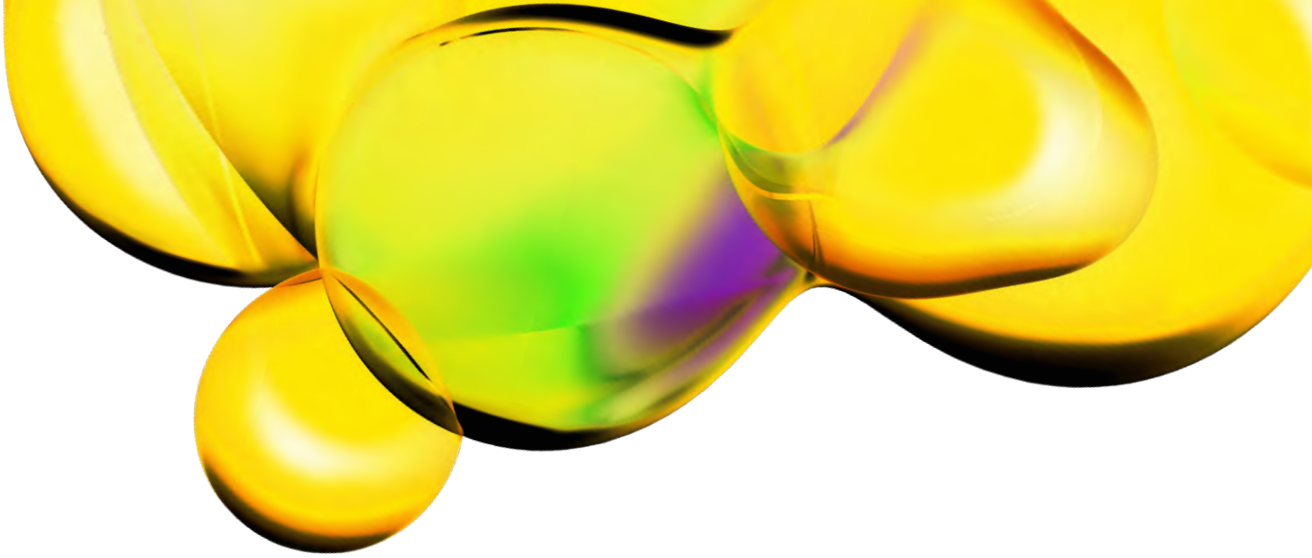
For more information about data reduction, please visit www.revvity.com

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)
Negative control	757	6.7%
Std 0	23655	3.4%
Std 1 - 0.0001 µM	21668	0.9%
Std 2 - 0.001 µM	21948	7.5%
Std 3 - 0.01 µM	15245	3.2%
Std 4 - 0.1 µM	5832	6.4%
Std 5 - 1 µM	1128	6.8%
Std 6 - 10 µM	770	2.1%
Std 7 - 100 µM	814	6.8%





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

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