

HUMAN H232 STING BINDING KITS

Part # 64BDSTGHPEG & 64BDSTGHPEH

Test size#: 500 tests (64BDSTGHPEG), 10,000 tests (64BDSTGHPEH) - assay volume: 20 μ L

Revision: #05 of September 2023

Store at: $\leq -60^{\circ}\text{C}$

For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

Revvity Human H232 STING binding assay is only intended for quantitative measurement of human H232 STING ligand using HTRF[®] technology.

Human H232 STING ligand is detected in a competitive assay format using a specific 6His antibody labeled with Terbium Cryptate (donor) which binds to human H232 STING protein 6His-tagged and H232 STING ligand labelled with d2 (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 H232 STING ligand labelled with d2, and thereby prevents FRET from occurring. The specific signal is inversely proportional to the compound concentration (Fig. 1).

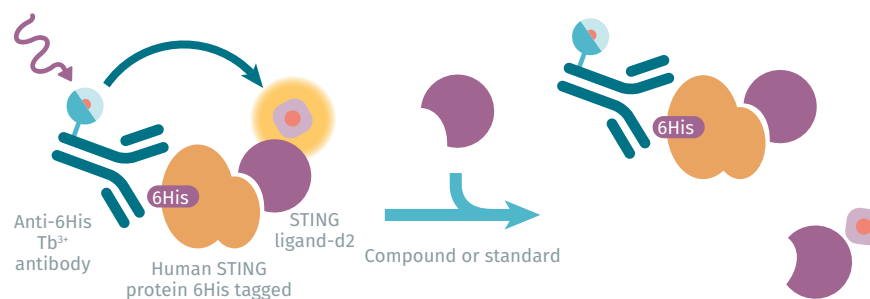
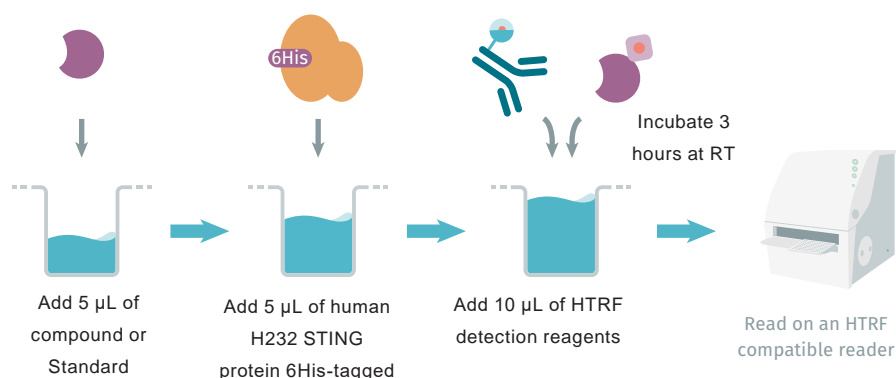


Figure 1: Principle of HTRF[®] Human H232 STING binding competitive assay.

MANUAL AT A GLANCE



Make sure you use the appropriate setup for Tb³⁺ 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 # 64BDSTGHPEG	10,000 tests * Cat # 64BDSTGHPEH
Human H232 STING binding kit - Standard Frozen - 10X	1 vial - 50 µL	2 vials - 50 µL
6His Tb Cryptate Antibody	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
H232 STING ligand d2 reagent	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
Human H232 STING protein 6His-tagged	2 vials - 25 µL Frozen - 50X	5 vials - 200 µL Frozen - 50X
Diluent #9 5X	3 vials 2 mL	1 vial 100 mL
Detection buffer #12 5X	1 vial 2 mL	1 vial 50 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.







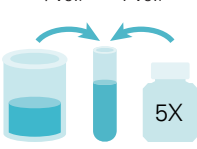
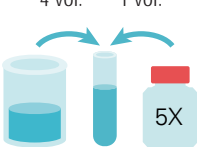
Thawed diluent and detection buffer can be stored at 2-8°C on your premises.

REAGENT PREPARATION**BEFORE YOU BEGIN:**

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Thaw protein on ice, other reagents can be thawed at room temperature
- Before use, allow diluent and buffer to warm up at room temperature and homogenize them with a vortex.
- Human H232 STING binding kit - Standard (for standard curve) must be prepared in diluent.

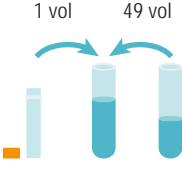
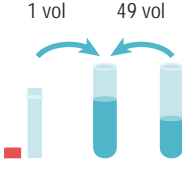
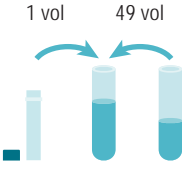
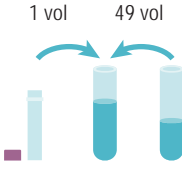
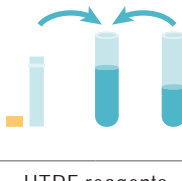

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64BDSTGHPEG			10,000 TESTS KIT - 64BDSTGHPEH
6His Cryptate antibody			
Thaw the 6His Tb Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.			Thaw the 6His Tb Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.
H232 STING ligand d2 reagent			
Thaw the H232 STING ligand d2 reagent. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.			Thaw the H232 STING ligand d2 reagent. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.
Human H232 STING binding kit - Standard			
Thaw the Human H232 STING binding kit - Standard. Centrifuge. This 10 X stock solution can be frozen and stored at -60°C or below.			Thaw the Human H232 STING binding kit - Standard. Centrifuge. This 10 X stock solution can be frozen and stored at -60°C or below.
human H232 STING protein 6His-tagged			
Thaw the human H232 STING protein 6His-tagged on ice. Centrifuge the vial. To avoid freeze/thaw cycles, it is recommended to aliquot the remainder of this 50X stock solution under 10 µL minimum in disposable plastic vials for storage at ≤-60°C.			Thaw the human H232 STING protein 6His-tagged on ice. Centrifuge the vial. To avoid freeze/thaw cycles, it is recommended to aliquot the remainder of this 50X stock solution under 10 µL minimum in disposable plastic vials for storage at ≤-60°C.
Diluent			
Dilute 5-fold the 5X diluent #9 with distilled water: Homogenize the 5X diluent #9 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water, e.g. 1 mL of diluent + 4 mL of distilled water. Mix gently after dilution.			Dilute 5-fold the 5X diluent #9 with distilled water: Homogenize the 5X diluent #9 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water, e.g. 10 mL of diluent + 40 mL of distilled water. Mix gently after dilution.
Detection Buffer			
Dilute 5-fold the 5X detection buffer #12 with distilled water: Homogenize the 5X detection buffer #12 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water, e.g. 1 mL of diluent + 4 mL of distilled water. Mix gently after dilution.			Dilute 5-fold the 5X detection buffer #12 with distilled water: Homogenize the 5X detection buffer #12 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water, e.g. 10 mL of diluent + 40 mL of distilled water. Mix gently after dilution.

TO PREPARE WORKING SOLUTIONS:

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

500 TESTS KIT - 64BDSTGHPEG			10,000 TESTS KIT - 64BDSTGHPEH
6His Tb Cryptate antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of 6His Tb cryptate antibody with detection buffer #12 (1X), eg 10 μ L of thawed Tb cryptate antibody stock solution + 490 μ L of detection buffer #12 (1X).			Dilute 50-fold the 50X stock solution (thawed reagent) of 6His Tb cryptate antibody with detection buffer #12 (1X), eg 10 μ L of thawed Tb cryptate antibody stock solution + 490 μ L of detection buffer #12 (1X).
H232 STING ligand d2 reagent			
Dilute 50-fold the 50X stock solution (thawed reagent) of H232 STING ligand d2 reagent with detection buffer #12 (1X), eg 10 μ L of thawed H232 STING ligand d2 reagent stock solution + 490 μ L of detection buffer #12 (1X).			Dilute 50-fold the 50X stock solution (thawed reagent) of H232 STING ligand d2 reagent with detection buffer #12 (1X), eg 10 μ L of thawed H232 STING ligand d2 reagent stock solution + 490 μ L of detection buffer #12 (1X).
human H232 STING protein 6His-tagged			
Dilute 50-fold the 50X stock solution (thawed reagent on ice) of human H232 STING 6His protein with detection buffer #12 (1X), eg 10 μ L of thawed protein stock solution + 490 μ L of detection buffer #12 (1X).			Dilute 50-fold the 50X stock solution (thawed reagent on ice) of human H232 STING 6His protein with detection buffer #12 (1X), eg 10 μ L of thawed protein stock solution + 490 μ L of detection buffer #12 (1X).
HTRF reagents			
It is possible to pre-mix the two ready-to-use solutions just prior to dispensing the reagents by adding 1 volume of H232 STING ligand d2 reagent solution to 1 volume of 6His Tb cryptate antibody solution (e.g. 1 mL of H232 STING ligand d2 reagent + 1 mL of 6His Tb cryptate antibody).			It is possible to pre-mix the two ready-to-use solutions just prior to dispensing the reagents by adding 1 volume of H232 STING ligand d2 reagent solution to 1 volume of 6His Tb cryptate antibody solution (e.g. 1 mL of H232 STING ligand d2 reagent + 1 mL of 6His Tb cryptate antibody).

TO PREPARE WORKING STANDARD SOLUTIONS:

- Each well requires 5 μL of standard.
- Dilute the standard stock solution serially with diluent #9.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

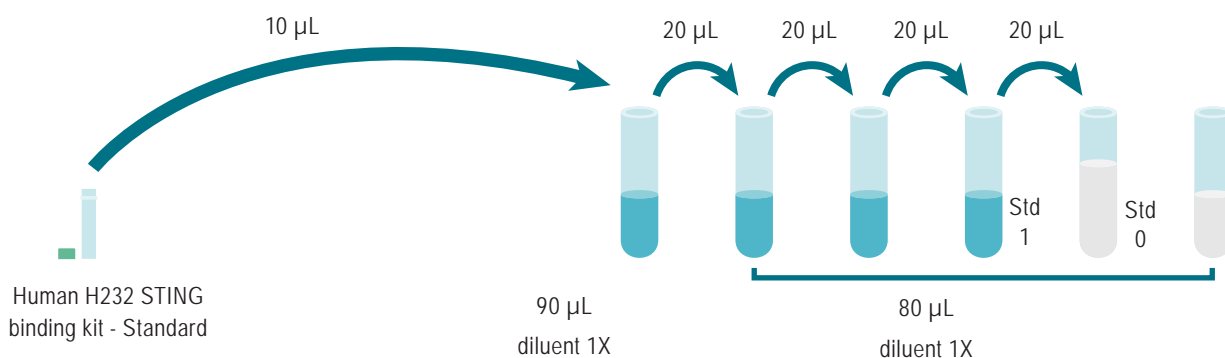
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 10-fold with diluent to prepare high standard (Std 7): take 10 μL of standard stock solution and add it to 90 μL of diluent #9 (1X). Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/5 serial dilutions as follows:

- Dispense 80 μL of diluent into each vial from Std 6 to Std 0.
- Add 20 μL of standard to 80 μL of diluent, mix gently and repeat the 1/5 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	HUMAN H232 STING STANDARD WORKING SOLUTION (nM)	HUMAN H232 STING STANDARD FINAL CONCENTRATION (nM)
Standard Stock solution	Thawed stock solution	200 000	
Standard 7	10 μL standard stock solution + 90 μL Diluent	20 000	5 000
Standard 6	20 μL standard 7 + 80 μL Diluent	4 000	1 000
Standard 5	20 μL standard 6 + 80 μL Diluent	800	200
Standard 4	20 μL standard 5 + 80 μL Diluent	160	40
Standard 3	20 μL standard 4 + 80 μL Diluent	32	8
Standard 2	20 μL standard 3 + 80 μL Diluent	6.4	1.6
Standard 1	20 μL standard 2 + 80 μL Diluent	1.28	0.32
Standard 0	100 μL Diluent	0	0

TO PREPARE SAMPLES:

- Each well requires 5 μL of compound.
- Dilute your compound in diluent #9 (1X).
- DMSO concentration must not exceed 2.5% final in the well (10% initial).

ASSAY MANUAL

		Negative control (or Cryptate control)	Standard (Std 0 - Std 7)	Compound
Step 1		Dispense 5 μ L of diluent into each negative control well.	Dispense 5 μ L of each Human H232 STING binding kit - Standard (Std 0 - Std 7) into each standard well.	Dispense 5 μ L of compound into each compound well.
Step 2		Add 5 μ L of detection buffer to all wells	Add 5 μ L of human H232 STING protein 6His-tagged protein to all wells	
Step 3		Add 10 μ L of premixed H232 STING ligand d2 reagent and 6His Tb antibody working solution to all wells		
Step 4		Seal the plate and incubate 3 hours at RT or at Over Night if necessary		
Step 5		Remove the plate sealer and read on an HTRF® compatible reader		

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.

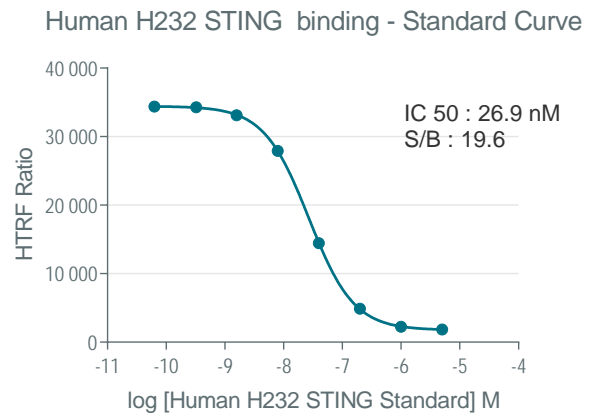
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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

RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example (readout on an HTRF compatible reader). Results may vary from one HTRF® compatible reader to another.

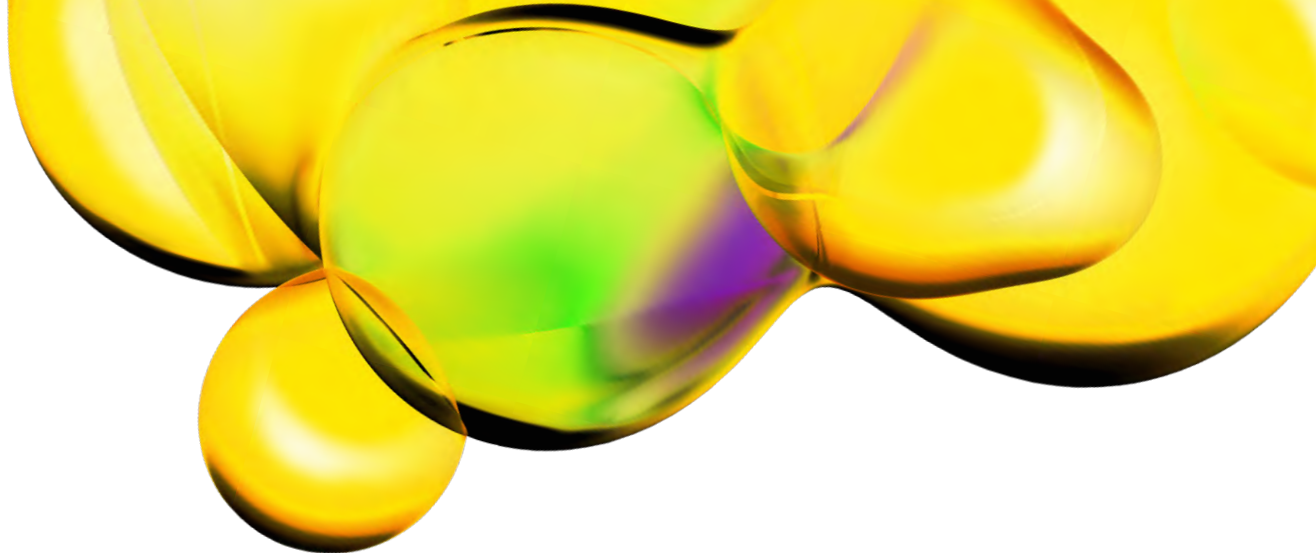
	Ratio ⁽¹⁾	CV ⁽²⁾
Negative control	1712	3.2%
Std 0	34368	2.0%
Std 1 - 0.32 nM	34286	1.8%
Std 2 - 1.6 nM	33122	1.3%
Std 3 - 8 nM	27930	1.0%
Std 4 - 40 nM	14457	2.1%
Std 5 - 200 nM	4891	2.2%
Std 6 - 1000 nM	2245	1.3%
Std 7 - 5000 nM	1844	3.1%



ANALYTICAL CHARACTERISTICS

Human H232 STING ligand-d2 Kd	20nM
Human H232 STING ligand-d2 concentration	20nM
Human H232 STING Standard IC50	26.9nM
Human H232 STING Standard Ki	13.4nM
Signal to background (S/B)	19.6

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