HUMAN H232 STING BINDING KITS

PROTOCOL

Part # 64BDSTGHPEG & 64BDSTGHPEH

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

Revision: 02 / Oct. 2019

Store at: -60°C or below

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

ASSAY PRINCIPLE

Cisbio Bioassays' 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 Anti 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).

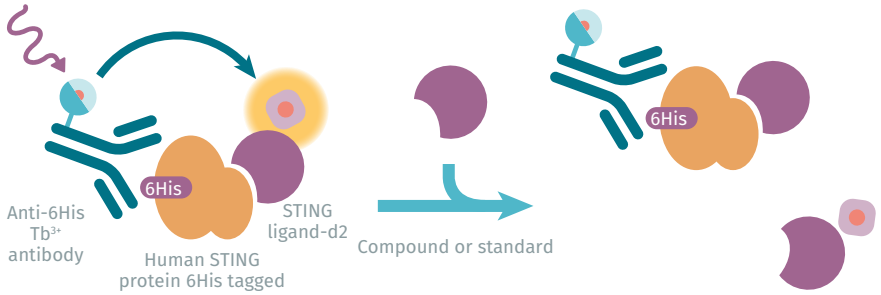
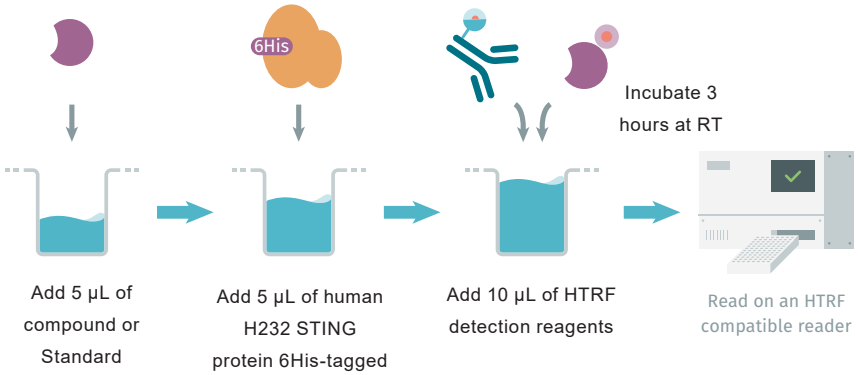


Figure 1: Principle of HTRF® Human H232 STING binding competitive assay.

PROTOCOL AT A GLANCE



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

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 Ref 64BDSTGHCDA	2 vials - 50 µL Ref 64BDSTGHCDA
Anti-6His-Tb^{3+} Cryptate Antibody	1 vial - 50 µL Frozen - 50 X	1 vial - 1 mL Frozen - 50 X
H232 STING ligand-d2	1 vial - 50 µL Frozen - 50 X	1 vial - 1 mL Frozen - 50 X
human H232 STING protein 6His-tagged	2 vials - 25 µL Frozen - 50 X	5 vials - 200 µL Frozen - 50 X
Diluent #9 5X	3 vials 2 mL	1 vial 100 mL (62DL9DDC)
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:

- HTRF® 96-well low volume plate Ref# 66PL96001 *
- HTRF® 384-well low volume plate Ref 66PL384025 *
- HTRF®-Certified Reader **. Make sure the setup for Tb^{3+} Cryptate is used.
- Use white plate only.

* For HTRF microplate recommendations, please visit http://www.cisbio.com/drug-discovery/htrf-microplate-recommendations
** For a list of HTRF-compatible readers and setup recommendations, please visit http://www.cisbio.com/compatible-readers

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.

Reagents

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.

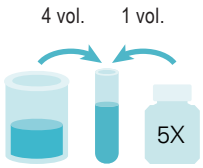
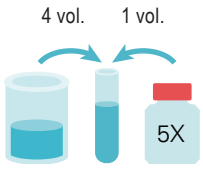
REAGENT PREPARATION

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.

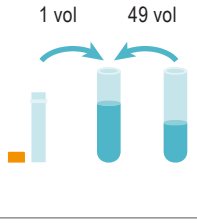
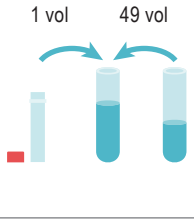
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64BDSTGHPEG		10,000 TESTS KIT - 64BDSTGHPEH
Anti-6His- Cryptate antibody		
Thaw the Anti-6His- Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.		Thaw the Anti-6His- Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.
H232 STING ligand-d2		
Thaw the H232 STING ligand-d2. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.		Thaw the H232 STING ligand-d2. 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 or below.		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 or below
Diluent		
Dilute 5-fold the 5 X diluent #9 with distilled water: Homogenize the 5 X 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 5 X diluent #9 with distilled water: Homogenize the 5 X 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 5 X detection buffer #12 with distilled water: Homogenize the 5 X 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 5 X detection buffer #12 with distilled water: Homogenize the 5 X 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 µL of each reagent.
Prepare in separate vials.

<table>
<tr><th colspan="2">500 TESTS KIT - 64BDSTGHPEG</th><th colspan="2">10,000 TESTS KIT - 64BDSTGHPEH</th></tr>
<tr><td colspan="4">Anti-6His- Cryptate antibody</td></tr>
<tr><td>Dilute 50-fold the 50X stock solution (thawed reagent) of anti-6His cryptate antibody with detection buffer #12 (1X), eg 10 µL of thawed cryptate-antibody stock solution + 490 µL of detection buffer #12 (1X).</td><td> 
</td><td> 
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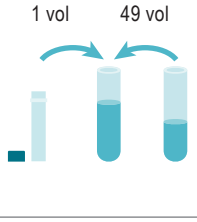
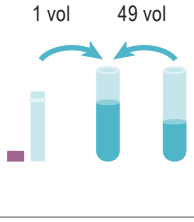
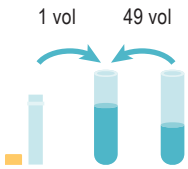
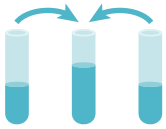
<tr><td colspan="4">H232 STING ligand-d2</td></tr>
<tr><td>Dilute 50-fold the 50X stock solution (thawed reagent) of H232 STING ligand-D2 with detection buffer #12 (1X), eg 10 µL of thawed H232 STING ligand-D2 stock solution + 490 µL of detection buffer #12 (1X).</td><td> 
</td><td> 
</td><td>Dilute 50-fold the 50X stock solution (thawed reagent) of H232 STING ligand-D2 with detection buffer #12 (1X), eg 10 µL of thawed H232 STING ligand-D2 stock solution + 490 µL of detection buffer #12 (1X).</td></tr>
<tr><td colspan="4">human H232 STING protein 6His-tagged</td></tr>
<tr><td>Dilute 50-fold the 50X stock solution (thawed reagent on ice) of human H232 STING 6His protein with detection buffer #12 (1X), eg 10 µL of thawed protein stock solution + 490 µL of detection buffer #12 (1X).</td><td colspan="2"> 
</td><td>Dilute 50-fold the 50X stock solution (thawed reagent on ice) of human H232 STING 6His protein with detection buffer #12 (1X), eg 10 µL of thawed protein stock solution + 490 µL of detection buffer #12 (1X).</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 H232 STING ligand-d2 solution to 1 volume of anti-6His cryptate antibody solution (e.g. 1 mL of H232 STING ligand-d2 + 1 mL of anti-6His 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 H232 STING ligand-d2 solution to 1 volume of anti-6His cryptate antibody solution (e.g. 1 mL of H232 STING ligand-d2 + 1 mL of anti-6His cryptate antibody).</td></tr>
</table>

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.

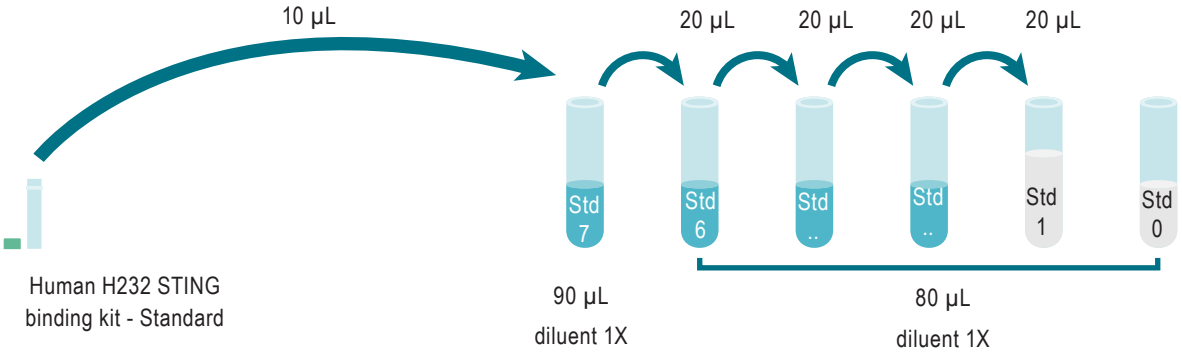
A recommended standard dilution procedure is listed and illustrated below:

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	10µl 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

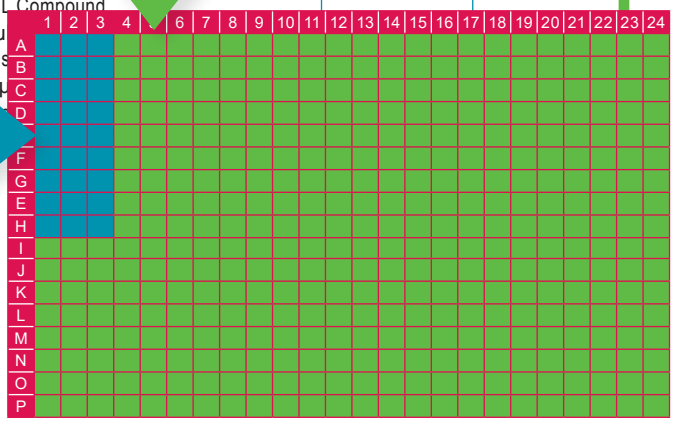
TO PREPARE SAMPLES:

- 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 PROTOCOL

	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 Human H232 STING binding kit - 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 H232 STING protein 6His-tagged protein to all wells	
Step 3 	Add 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed 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		

	1	2	3	4	5	6
A	5 µL Diluent (Negative control) 5 µL of detection buffer 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well A1	Repeat Well A1	5 µL Compound 1 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well A4	Repeat Well A4
B	5 µL Std 0 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well B1	Repeat Well B1	5 µL Compound 2 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well B4	Repeat Well B4
C	5 µL Std 1 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well C1	Repeat Well C1	5 µL Compound 3 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well C4	Repeat Well C4
D	5 µL Std ... 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well D1	Repeat Well D1	5 µL Compound ... 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well D4	Repeat Well D4
E	5 µL Std ... 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well E1	Repeat Well E1	5 µL Compound ... 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well E4	Repeat Well E4
F	5 µL Std ... 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well F1	Repeat Well F1	5 µL Compound ... 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well F4	Repeat Well F4
G	5 µL Std ... 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well G1	Repeat Well G1	5 µL Compound ... 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premix	Repeat Well G4	Repeat Well G4
H	5 µL Std ... 5 µL of human H232 STING protein 6His-tagged 10 µL of H232 STING ligand-d2 and Anti 6His-Tb^{3+} premixed	Repeat Well H1	Repeat Well H1	5 µL Compound 5 µ 6His 10 µ		



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.

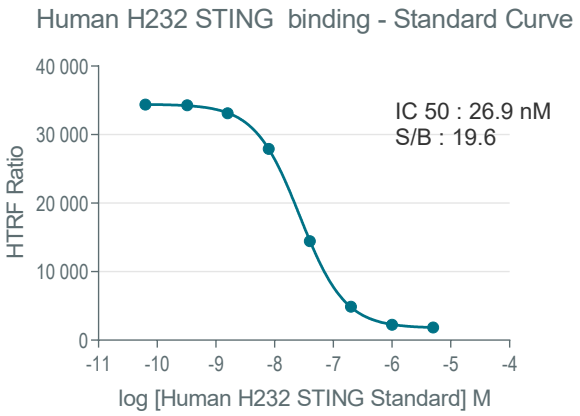
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit http://www.cisbio.com/htrf-ratio-and-data-reduction

RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example (readouts on Pherastar FS with a flash lamp). 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

FOR MORE INFORMATION
Europe and other countries +33(0)466-796-705 U.S. and Canada 1-888-963-4567 China +86-21-5018-9880
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INTERACTION IS EVERYTHING