MOUSE STING WT BINDING KITS

PROTOCOL

Part # 64BDSTGMPEG & 64BDSTGMPEH

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

Revision: 03 / May 2020

Store at: ≤-60°C

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

ASSAY PRINCIPLE

Cisbio Bioassays' Mouse STING WT binding assay is only intended for quantitative measurement of Mouse STING WT ligand using HTRF® technology.

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

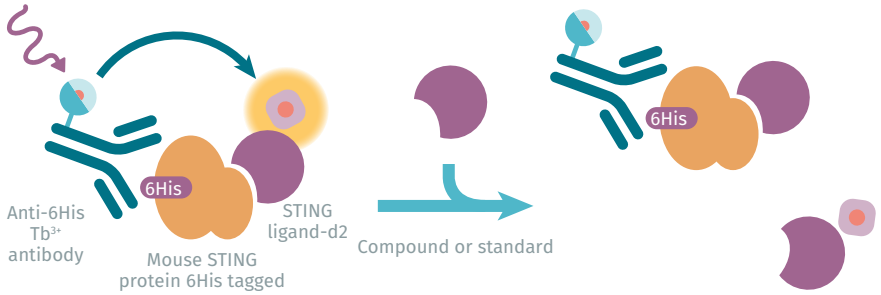
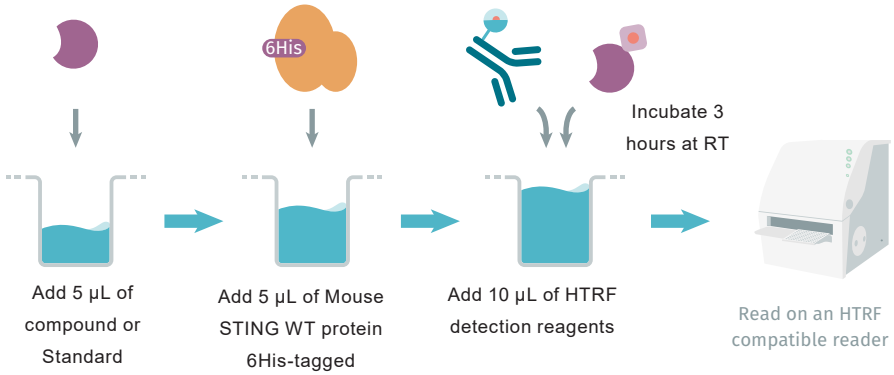


Figure 1: Principle of HTRF® Mouse STING WT binding competitive assay.

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

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MATERIALS PROVIDED:

Kit components	500 tests * Cat # 64BDSTGMPEG	10,000 tests * Cat # 64BDSTGMPEH
Mouse STING WT 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
Mouse STING WT ligand d2 reagent	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
Mouse STING WT 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 Tb^{3+} Cryptate is used.

* For HTRF microplate recommendations, please visit www.cisbio.com/microplate-recommendations

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

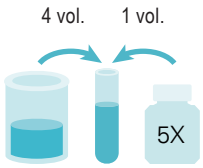
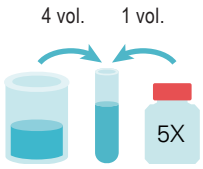
REAGENT PREPARATION

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.
- Mouse STING WT binding kit - Standard (for standard curve) must be prepared in diluent.

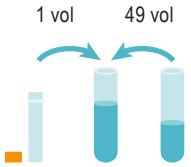
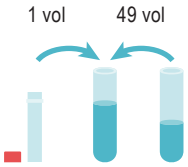
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64BDSTGMPEG		10,000 TESTS KIT - 64BDSTGMPEH
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.
Mouse STING WT ligand d2 reagent		
Thaw the Mouse STING WT ligand d2 reagent. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.		Thaw the Mouse STING WT ligand d2 reagent. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.
Mouse STING WT binding kit - Standard		
Thaw the Mouse STING WT binding kit - Standard. Centrifuge. This 10 X stock solution can be frozen and stored at -60°C or below.		Thaw the Mouse STING WT binding kit - Standard. Centrifuge. This 10 X stock solution can be frozen and stored at -60°C or below.
Mouse STING WT protein 6His-tagged		
Thaw the Mouse STING WT 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 Mouse STING WT 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 µL of each reagent.
Prepare in separate vials.

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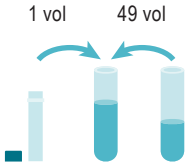
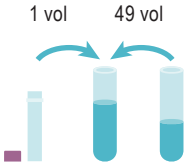
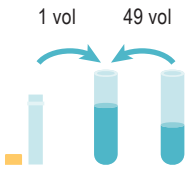
<tr><td>Dilute 50-fold the 50X stock solution (thawed reagent) of Mouse STING WT ligand d2 reagent with detection buffer #12 (1X), eg 10 µL of thawed Mouse STING WT ligand d2 reagent stock solution + 490 µL of detection buffer #12 (1X).</td><td> 
</td><td> 
</td><td>Dilute 50-fold the 50X stock solution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

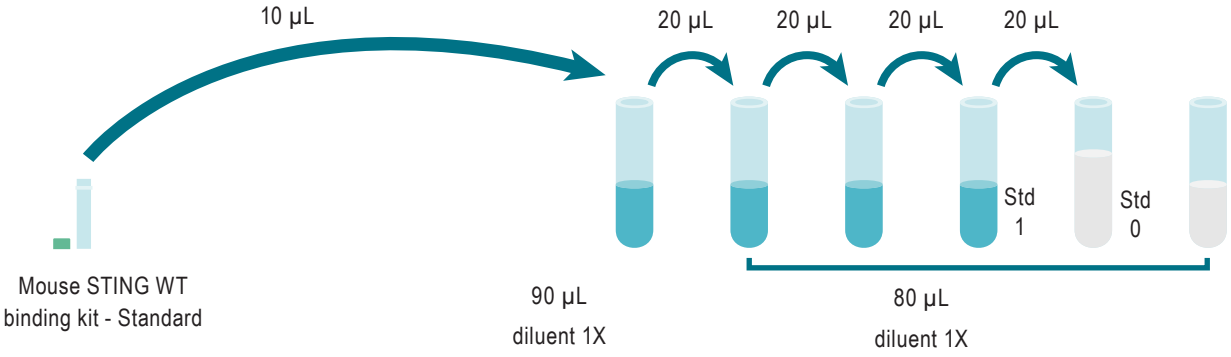
A recommended standard dilution procedure is listed and illustrated below:

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	MOUSE STING WT STANDARD WORKING SOLUTION (nM)	MOUSE STING WT STANDARD FINAL CONCENTRATION (nM)
Standard Stock solution	Thawed stock solution	2 000	
Standard 7	10µl standard stock solution + 90 µL Diluent	200	50
Standard 6	20 µL standard 7 + 80 µL Diluent	40	10
Standard 5	20 µL standard 6 + 80 µL Diluent	8	2
Standard 4	20 µL standard 5 + 80 µL Diluent	2	0
Standard 3	20 µL standard 4 + 80 µL Diluent	0.32	0.08
Standard 2	20 µL standard 3 + 80 µL Diluent	0.064	0.016
Standard 1	20 µL standard 2 + 80 µL Diluent	0.0128	0.0032
Standard 0	100 µL Diluent	0	0

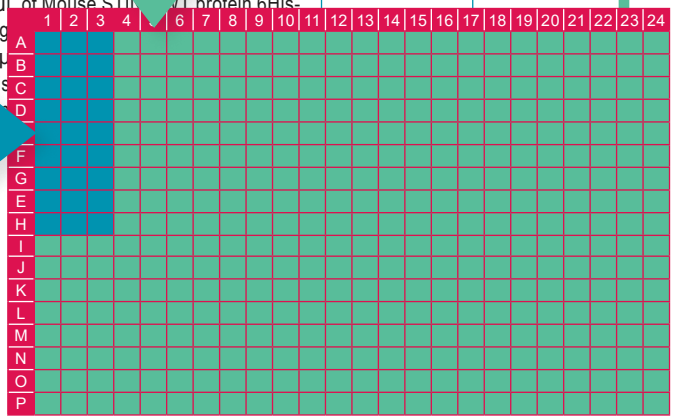
TO PREPARE SAMPLES:

- 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 Mouse STING WT 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 Mouse STING WT protein 6His-tagged protein to all wells	
Step 3 	Add 10 µL of premixed Mouse STING WT 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		

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



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.

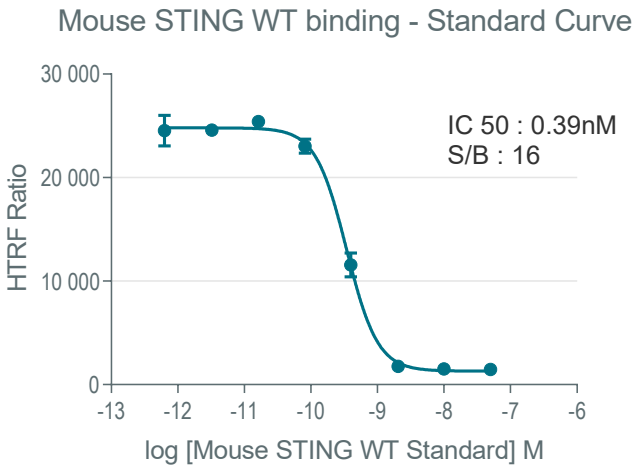
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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

	Ratio (1)	CV (2)
Negative control	1402	3.2%
Std 0	24543	6.1%
Std 1 - 0.004 nM	24598	1.5%
Std 2 - 0.016 nM	25417	1.6%
Std 3 - 0.08 nM	23028	3.2%
Std 4 - 0.4 nM	11562	9.4%
Std 5 - 2 nM	1763	4.2%
Std 6 - 10 nM	1503	1.3%
Std 7 - 50 nM	1451	3.5%



ANALYTICAL CHARACTERISTICS

Mouse STING WT ligand-d2 Kd	1.5nM
Mouse STING WT ligand-d2 concentration	3nM
Mouse STING WT Standard IC50	0.39nM
Mouse STING WT Standard Ki	0.13nM
Signal to background (S/B)	16

FOR MORE INFORMATION
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Japan +81-(0)43-306-8712 Visit www.cisbio.com to find a list of our regional distributors

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