



HUMAN STING WT BINDING KITS

PROTOCOL

Part # 64BDSTGPEG & 64BDSTGPEH

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

Revision: 05 / May 2020

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

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

ASSAY PRINCIPLE

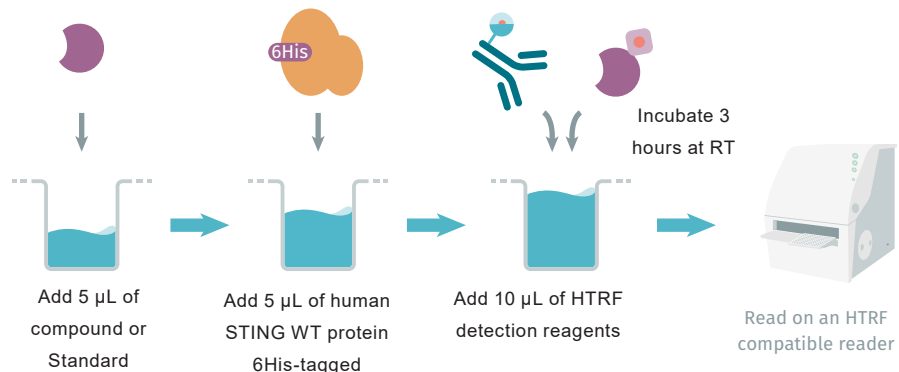
Cisbio Bioassays' human STING WT binding assay is only intended for quantitative measurement of human STING WT ligand using HTRF[®] technology.

Human STING WT ligand is detected in a competitive assay format using a specific 6His antibody labeled with Terbium Cryptate (donor) which binds to human STING WT protein 6His-tagged and 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 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[®] human STING WT binding competitive assay.

PROTOCOL 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: <http://www.cisbio.com/compatible-readers>

MATERIALS PROVIDED:

Kit components	500 tests * Cat # 64BDSTGPEG	10,000 tests * Cat # 64BDSTGPEH
Human STING WT Standard 2'3'-cGAMP Frozen - 10X	1 vial - 50 μ L	2 vials - 50 μ L
6His Tb Cryptate Antibody	1 vial - 50 μ L Frozen - 50X	1 vial - 1 mL Frozen - 50X
STING WT ligand d2 reagent	1 vial - 50 μ L Frozen - 50X	1 vial - 1 mL Frozen - 50X
human STING WT protein 6His-tagged	2 vials - 25 μ L Frozen - 50X	5 vials - 200 μ L 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 μ L 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.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 μ L.







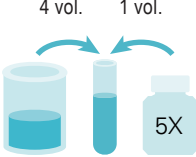
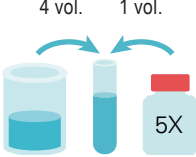
Thawed diluent and detection buffer can be stored at 2-8°C on your premises.

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 STING WT Standard 2'3'-cGAMP (for standard curve) must be prepared in diluent.

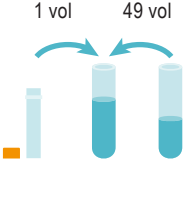
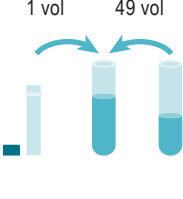

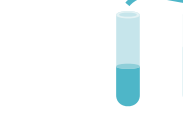
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64BDSTGPEG			10,000 TESTS KIT - 64BDSTGPEH
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.
STING WT ligand d2 reagent			
Thaw the STING WT ligand d2 reagent. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.			Thaw the STING WT ligand d2 reagent. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.
Human STING WT Standard 2'3'-cGAMP			
Thaw the Human STING WT Standard 2'3'-cGAMP. Centrifuge. This 10 X stock solution can be frozen and stored at -60°C or below.			Thaw the Human STING WT Standard 2'3'-cGAMP. Centrifuge. This 10 X stock solution can be frozen and stored at -60°C or below.
human STING WT protein 6His-tagged			
Thaw the human 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 human 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 μ L of each reagent.
Prepare in separate vials.

500 TESTS KIT - 64BDSTGPEG	10,000 TESTS KIT - 64BDSTGPEH	
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).
STING WT ligand d2 reagent		
Dilute 50-fold the 50X stock solution (thawed reagent) of STING WT ligand d2 reagent with detection buffer #12 (1X), eg 10 μ L of thawed STING WT ligand d2 reagent stock solution + 490 μ L of detection buffer #12 (1X).		Dilute 50-fold the 50X stock solution (thawed reagent) of STING WT ligand d2 reagent with detection buffer #12 (1X), eg 10 μ L of thawed STING WT ligand d2 reagent stock solution + 490 μ L of detection buffer #12 (1X).
human STING WT protein 6His-tagged		
Dilute 50-fold the 50X stock solution (thawed reagent on ice) of human STING WT 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 STING WT 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 STING WT ligand d2 reagent solution to 1 volume of 6His Tb cryptate antibody solution (e.g. 1 mL of STING WT 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 STING WT ligand d2 reagent solution to 1 volume of 6His Tb cryptate antibody solution (e.g. 1 mL of STING WT 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.

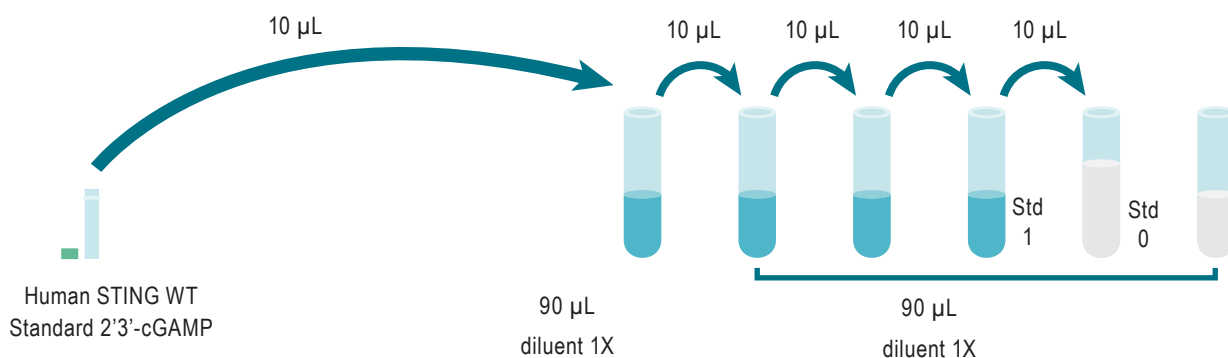
A recommended standard dilution procedure is listed and illustrated below:

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 1/10 serial dilutions as follows:

- Dispense 90 μL of diluent into each vial from Std 6 to Std 0.
- Add 10 μL of standard to 90 μL of diluent, 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 diluent alone.








STANDARD	SERIAL DILUTIONS	HUMAN STING WT STANDARD 2'3'-CGAMP WORKING SOLUTION (nM)	HUMAN STING WT STANDARD 2'3'-CGAMP 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	10 μL standard 7 + 90 μL Diluent	2 000	500
Standard 5	10 μL standard 6 + 90 μL Diluent	200	50
Standard 4	10 μL standard 5 + 90 μL Diluent	20	5
Standard 3	10 μL standard 4 + 90 μL Diluent	2	0.5
Standard 2	10 μL standard 3 + 90 μL Diluent	0.2	0.05
Standard 1	10 μL standard 2 + 90 μL Diluent	0.02	0.005
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 μ L of diluent into each negative control well.	Dispense 5 μ L of each Human STING WT Standard 2'3'-cGAMP (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 STING WT protein 6His-tagged protein to all wells	
Step 3 		Add 10 μ L of premixed 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		

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.

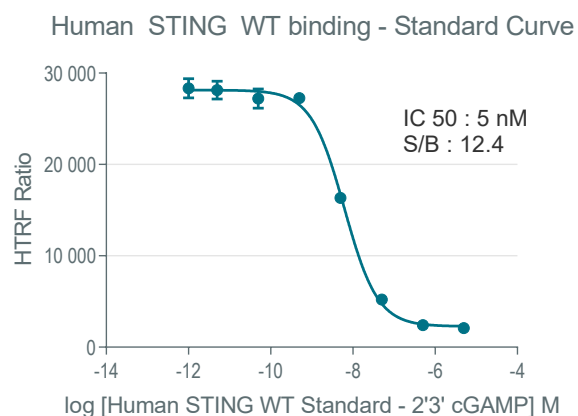
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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	2042	1.8%
Std 0	28330	3.7%
Std 1 - 0.005 nM	28128	3.5%
Std 2 - 0.05 nM	27218	3.9%
Std 3 - 0.5 nM	27274	0.6%
Std 4 - 5 nM	16349	1.0%
Std 5 - 50 nM	5218	6.3%
Std 6 - 500 nM	2422	2.1%
Std 7 - 5000 nM	2076	5.1%



ANALYTICAL CHARACTERISTICS

STING WT ligand-d2 Kd	15nM
STING WT ligand-d2 concentration	15nM
Human STING WT Standard 2'3'-cGAMP IC ₅₀	5nM
Human STING WT Standard 2'3'-cGAMP Ki	2.5nM
Signal to background (S/B)	12.4

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