



HTRF HUMAN CEREBLON BINDING KITS

PROTOCOL

Part # 64BDCRBNPEG & 64BDCRBNPEH

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

Revision: 02 / May 2021

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

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

ASSAY PRINCIPLE

PerkinElmer' Cereblon binding assay is only intended for quantitative measurement of Cereblon WT ligands using HTRF[®] technology.

Cereblon WT ligands are detected in a competitive assay format using a specific GST antibody labeled with Europium Cryptate (donor) which binds to Human Cereblon WT GST-tagged and Thalidomide-Red labelled with XL665 (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 Thalidomide-Red labelled with XL665, and thereby prevents FRET from occurring. The specific signal is inversely proportional to the compound concentration (Fig. 1).

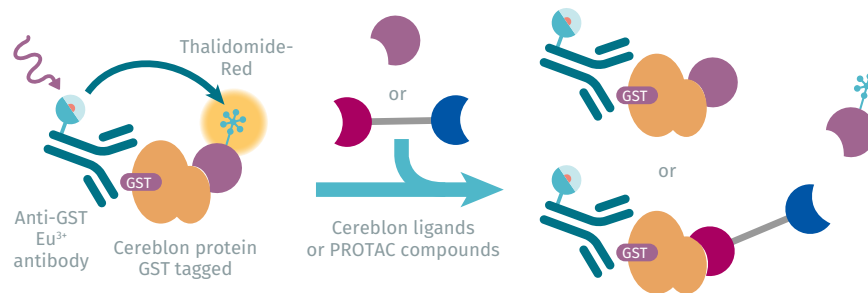
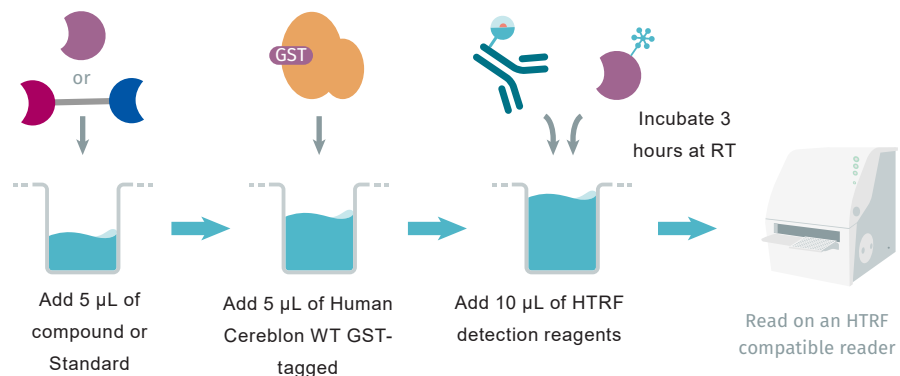


Figure 1: Principle of HTRF[®] Cereblon binding competitive assay.

PROTOCOL AT A GLANCE



Make sure you use the appropriate setup for Eu³⁺ 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 # 64BDCRBNPEG	10,000 tests * Cat # 64BDCRBNPEH
Cereblon binding standard Frozen - 10X	1 vial - 30 μ L	2 vials - 30 μ L
GST Eu Cryptate Antibody	1 vial - 50 μ L Frozen - 50X	1 vial - 1 mL Frozen - 50X
Thalidomide-Red reagent	1 vial - 50 μ L Frozen - 50X	1 vial - 1 mL Frozen - 50X
Human Cereblon WT GST-tagged	2 vials - 30 μ L Frozen - 45X	4 vials - 280 μ L Frozen - 45X
Diluent #9 5X	3 vials 2 mL	1 vial 100 mL
PROTAC binding buffer 1 1X	1 vial 20 mL	1 vial 200 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 Eu³⁺ 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.





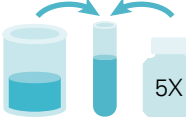
Thawed diluent and detection buffer can be stored at 2-8°C on your premises.

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.
- Cereblon binding standard (for standard curve) must be prepared in diluent. Cereblon binding standard is the E3 ligase ligand : Lenalidomide.

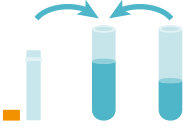
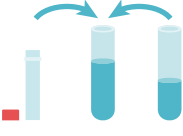
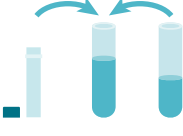
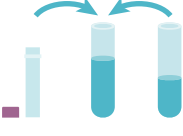
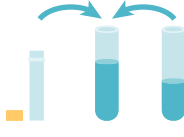

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64BDCRBNPEG	10,000 TESTS KIT - 64BDCRBNPEH	
GST Cryptate antibody		
Thaw the GST Eu Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.		Thaw the GST Eu Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.
Thalidomide-Red reagent		
Thaw the Thalidomide-Red reagent. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.		Thaw the Thalidomide-Red reagent. Centrifuge. This 50X stock solution can be frozen and stored at -60°C or below.
Cereblon binding standard		
Thaw the Cereblon binding standard. Centrifuge. This 10 X stock solution can be frozen and stored at -60°C or below.		Thaw the Cereblon binding standard. Centrifuge. This 10 X stock solution can be frozen and stored at -60°C or below.
Human Cereblon WT GST-tagged		
Thaw the Human Cereblon WT GST-tagged on ice. Centrifuge the vial. To avoid freeze/thaw cycles, it is recommended to aliquot the remainder of this 45X stock solution under 10 µL minimum in disposable plastic vials for storage at ≤-60°C.		Thaw the Human Cereblon WT GST-tagged on ice. Centrifuge the vial. To avoid freeze/thaw cycles, it is recommended to aliquot the remainder of this 45X 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.	<p>4 vol. 1 vol.</p> 	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.

TO PREPARE WORKING SOLUTIONS:

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

500 TESTS KIT - 64BDCRBNPEG			10,000 TESTS KIT - 64BDCRBNPEH
GST Eu Cryptate antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of GST Eu cryptate antibody with PROTAC binding buffer 1 (1X), eg 10 μ L of thawed Eu cryptate antibody stock solution + 490 μ L of PROTAC binding buffer 1 (1X).			Dilute 50-fold the 50X stock solution (thawed reagent) of GST Eu cryptate antibody with PROTAC binding buffer 1 (1X), eg 10 μ L of thawed Eu cryptate antibody stock solution + 490 μ L of PROTAC binding buffer 1 (1X).
Thalidomide-Red reagent			
Dilute 50-fold the 50X stock solution (thawed reagent) of Thalidomide-Red reagent with PROTAC binding buffer 1 (1X), eg 10 μ L of thawed Thalidomide-Red reagent stock solution + 490 μ L of PROTAC binding buffer 1 (1X).			Dilute 50-fold the 50X stock solution (thawed reagent) of Thalidomide-Red reagent with PROTAC binding buffer 1(1X), eg 10 μ L of thawed Thalidomide-Red reagent stock solution + 490 μ L of PROTAC binding buffer 1 (1X).
Human Cereblon WT GST-tagged			
Dilute 45-fold the 45X stock solution (thawed reagent on ice) of Human Cereblon WT GST-tagged protein with PROTAC binding buffer 1 (1X), eg 10 μ L of thawed protein stock solution + 440 μ L of PROTAC binding buffer 1 (1X).			Dilute 45-fold the 45X stock solution (thawed reagent on ice) of Human Cereblon WT GST-tagged protein with PROTAC binding buffer 1 (1X), eg 10 μ L of thawed protein stock solution + 440 μ L of PROTAC binding buffer 1 (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 Thalidomide-Red reagent solution to 1 volume of GST Eu cryptate antibody solution (e.g. 1 mL of Thalidomide-Red reagent + 1 mL of GST Eu 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 Thalidomide-Red reagent solution to 1 volume of GST Eu cryptate antibody solution (e.g. 1 mL of Thalidomide-Red reagent + 1 mL of GST Eu 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.

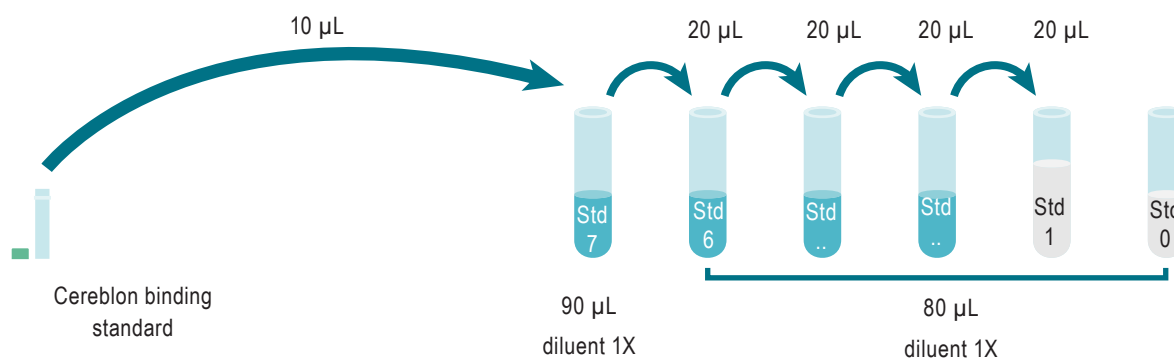
A recommended standard dilution procedure is listed and illustrated below:

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	"CEREBLON BINDING STANDARD WORKING SOLUTION (μM)"	"CEREBLON BINDING STANDARD FINAL CONCENTRATION (μM)"
Standard Stock solution	Thawed stock solution	8 000	
Standard 7	10 μL standard stock solution + 90 μL Diluent	800	200
Standard 6	20 μL standard 7 + 80 μL Diluent	160	40
Standard 5	20 μL standard 6 + 80 μL Diluent	32	8
Standard 4	20 μL standard 5 + 80 μL Diluent	6.4	1.6
Standard 3	20 μL standard 4 + 80 μL Diluent	1.28	0.32
Standard 2	20 μL standard 3 + 80 μL Diluent	0.256	0.064
Standard 1	20 μL standard 2 + 80 μL Diluent	0.051	0.0128
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% final in the well (fold of change is impaired by increasing percentage of DMSO).

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 Cereblon binding 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 PROTAC binding buffer 1 to all wells	Add 5 μ L of Human Cereblon WT GST-tagged protein to all wells	
Step 3		Add 10 μ L of premixed Thalidomide-Red reagent and GST Eu antibody working solution to all wells		
Step 4		Seal the plate and incubate 3 hours at RT		
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.

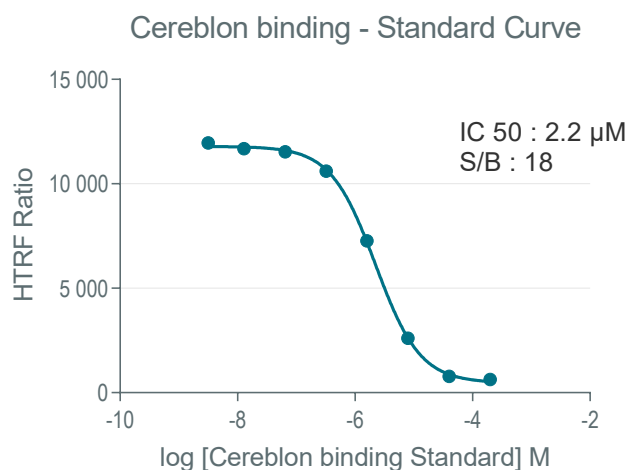
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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	678	2.0%
Std 0	11961	1.0%
Std 1 - 0.0128 µM	11677	1.2%
Std 2 - 0.064 µM	11527	0.7%
Std 3 - 0.32 µM	10611	0.4%
Std 4 - 1.6 µM	7268	1.1%
Std 5 - 8 µM	2607	1.0%
Std 6 - 40 µM	777	5.0%
Std 7 - 200 µM	631	5.8%



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