



HTRF **Human** MDM2 Binding Kit

Part # 64BDMDM2PEG & 64BDMDM2PEH

Test Size#: 500 TESTS (64BDMDM2PEG), 10,000 TESTS (64BDMDM2PEH) - assay volume: 20 μ L

Revision: 02 of September 2023 **Store at:** $\leq -60^{\circ}\text{C}$

This product is intended for research purposes only. It is not intended to be used for therapeutic or diagnostic purposes.

ASSAY PRINCIPLE

Revvity' HTRF MDM2 Binding assay is only intended for quantitative measurement of MDM2 ligands using HTRF[®] technology.

MDM2 ligands are detected in a competitive assay format using a specific GST antibody labeled with Europium Cryptate (donor) which binds to Human MDM2 WT GST-tagged protein and HTRF MI-1061 Red Ligand labelled with Red (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 HTRF MI-1061 Red Ligand, and thereby prevents FRET from occurring. The specific signal is inversely proportional to the compound concentration (Fig. 1)

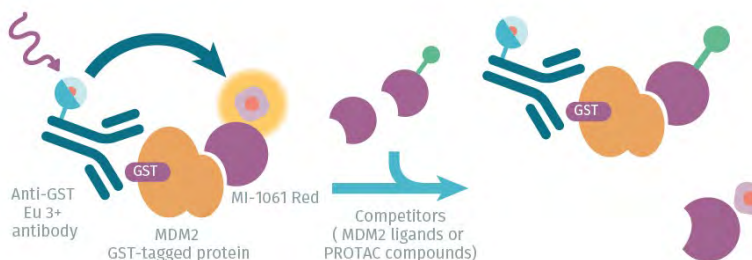
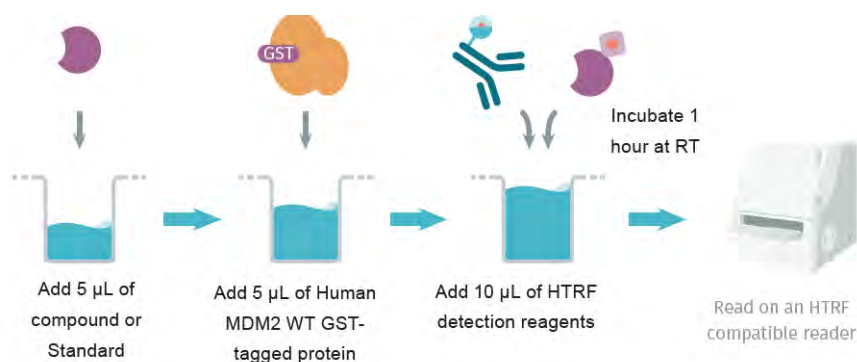


Figure 1: Principle of HTRF[®] MDM2 binding competitive assay.

MANUAL 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: www.revvity.com

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS* CAT # 64BDMDM2PEG	10,000 TESTS* CAT # 64BDMDM2PEH
HTRF MDM2 Binding Kit - 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
HTRF MI-1061 Red Ligand	1 vial - 50 μ L Frozen - 50X	1 vial - 1 mL Frozen - 50X
Human MDM2 WT GST-tagged protein	1 vial - 50 μ L Frozen - 50X	1 vial - 1 mL Frozen - 50X
Diluent #9 5X	3 vials 2 mL	1 vial 100 mL
PROTAC binding buffer 3 1X	1 vial 20 mL	1 vial 220 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.revity.com

** For a list of HTRF-compatible readers and setup recommendations, please visit www.revity.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. Freeze rapidly (do not keep at 4°C or at room temperature)

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







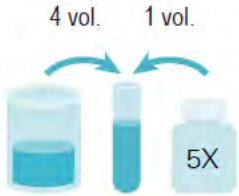

Working solutions should be prepared the day of the experiment and not stored for longer period.

REAGENT PREPARATION**BEFORE YOU BEGIN:**

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent or Binding buffer may affect reagent stability and assay results.
- Thaw protein on ice, other reagents can be thawed at room temperature
- Before use, allow buffer to warm up at room temperature and homogenize it with a vortex.
- HTRF MDM2 Binding Kit - Standard (for standard curve) must be prepared in diluent #9. MDM2 binding Standard is the AMG 232 compound.

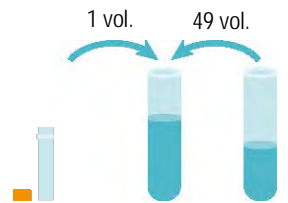
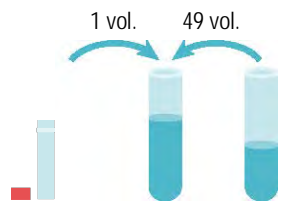
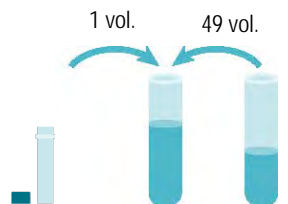
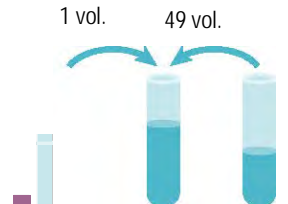


TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64BDMDM2PEG		10,000 TESTS KIT - 64BDMDM2PEH	
GST Eu Cryptate antibody			
Thaw the GST Eu Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.			Thaw the GST Eu Cryptate antibody. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.
MI-1061 Red ligand			
Thaw the MI-1061 Red ligand. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.			Thaw the MI-1061 Red ligand. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.
HTRF MDM2 Binding Kit - Standard			
Thaw the HTRF MDM2 Binding Kit - Standard. Centrifuge. This 10 X stock solution can be frozen and stored at -16°C or below.			Thaw the HTRF MDM2 Binding Kit - Standard. Centrifuge. This 10 X stock solution can be frozen and stored at -16°C or below.
Human MDM2 WT GST-tagged protein			
Thaw the Human MDM2 WT GST-tagged on ice. Centrifuge the vial. To avoid freeze/thaw cycles, it is recommended to aliquot the remainder of this 50X stock solution under 20 µL minimum in disposable plastic vials for storage at ≤-60°C.			Thaw the Human MDM2 WT GST-tagged on ice. Centrifuge the vial. To avoid freeze/thaw cycles, it is recommended to aliquot the remainder of this 50X stock solution under 20 µL minimum in disposable plastic vials for storage at ≤-60°C.
Diluent #9			
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.
PROTAC Binding Buffer 3			
Ready to use.			Ready to use.

TO PREPARE WORKING SOLUTIONS:

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

500 TESTS KIT - 64BDMDM2PEG		10,000 TESTS KIT - 64BDMDM2PEH	
GST Eu Cryptate antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of GST Eu cryptate antibody with PROTAC binding buffer 3 (1X), eg 10 μ L of thawed Eu cryptate antibody stock solution + 490 μ L of PROTAC binding buffer 3 (1X).			Dilute 50-fold the 50X stock solution (thawed reagent) of GST Eu cryptate antibody with PROTAC binding buffer 3 (1X), eg 10 μ L of thawed Eu cryptate antibody stock solution + 490 μ L of PROTAC binding buffer 3 (1X).
MI-1061 Red ligand			
Dilute 50-fold the 50X stock solution (thawed reagent) of MI-1061 Red Ligand with PROTAC binding buffer 3 (1X), eg 10 μ L of thawed MI-1061 Red Ligand stock solution + 490 μ L of PROTAC binding buffer 3 (1X).			Dilute 50-fold the 50X stock solution (thawed reagent) of MI-1061 Red Ligand with PROTAC binding buffer 3 (1X), eg 10 μ L of thawed MI-1061 Red Ligand stock solution + 490 μ L of PROTAC binding buffer 3 (1X).
Human MDM2 WT GST-tagged protein			
Dilute 50-fold the 50X stock solution (thawed reagent on ice) of MDM2 GST-Tagged protein with PROTAC binding buffer 3 (1X), eg 10 μ L of thawed protein stock solution + 490 μ L of PROTAC binding buffer 3 (1X).			Dilute 50-fold the 50X stock solution (thawed reagent on ice) of MDM2 GST-Tagged protein with PROTAC binding buffer 3 (1X), eg 10 μ L of thawed protein stock solution + 490 μ L of PROTAC binding buffer 3 (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 MI-1061 Red Ligand solution to 1 volume of GST Eu cryptate antibody solution (e.g. 1 mL of MI-1061 Red Ligand + 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 MI-1061 Red Ligand solution to 1 volume of GST Eu cryptate antibody solution (e.g. 1 mL of MI-1061 Red Ligand + 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.

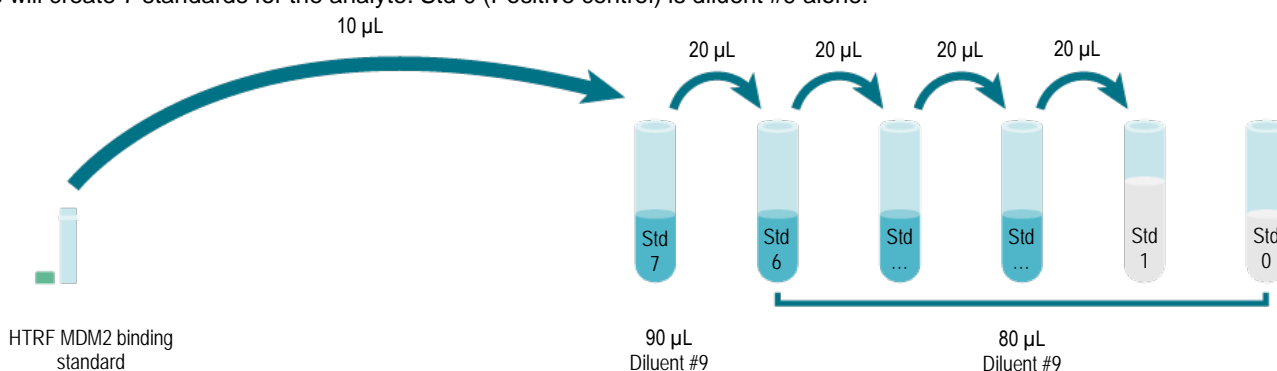
A recommended standard dilution procedure is listed and illustrated below:

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 #9 into each vial from Std 6 to Std 0.
- Add 20 μL of standard to 80 μL of diluent #9, 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 #9 alone.








STANDARD	SERIAL DILUTIONS	"HTRF MDM2 BINDING KIT-STANDARD WORKING SOLUTION (nM)"	" HTRF MDM2 BINDING KIT-STANDARD FINAL CONCENTRATION (nM)"
Standard Stock solution	Thawed stock solution	80 000	-
Standard 7	10 μL standard stock solution + 90 μL Diluent #9 (1X)	8 000	2 000
Standard 6	20 μL standard 7 + 80 μL Diluent #9 (1X)	1 600	400
Standard 5	20 μL standard 6 + 80 μL Diluent #9 (1X)	320	80
Standard 4	20 μL standard 5 + 80 μL Diluent #9 (1X)	64	16
Standard 3	20 μL standard 4 + 80 μL Diluent #9 (1X)	12.8	3.2
Standard 2	20 μL standard 3 + 80 μL Diluent #9 (1X)	2.56	0.64
Standard 1	20 μL standard 2 + 80 μL Diluent #9 (1X)	0.512	0.128
Standard 0	80 μL Diluent #9 (1X)	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 MANUAL

	NEGATIVE CONTROL (OR CRYPTATE CONTROL)	STANDARD (STD 0 - STD 7)	COMPOUND
Step 1 	Dispense 5 μ L of diluent #9 into each negative control well.	Dispense 5 μ L of each HTRF MDM2 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 PROTAC Binding buffer 3 to all wells	Add 5 μ L of Human MDM2 WT GST-tagged protein to all wells	
Step 3 	Add 10 μ L of premixed HTRF MI-1061 Red Ligand and GST Eu Cryptate antibody working solution to all wells		
Step 4 	Seal the plate and incubate 1 hour 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.

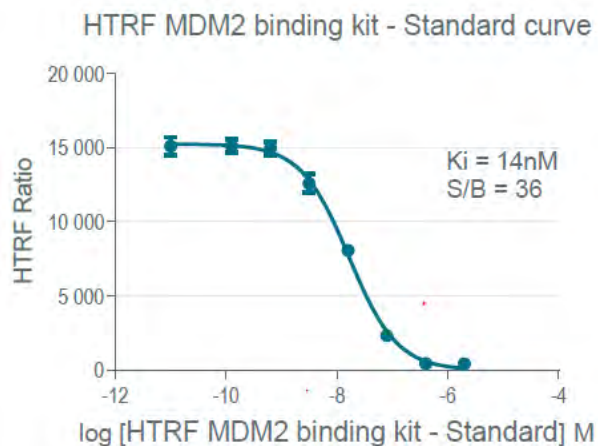
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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

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	405	3.0%
Std 0	15105	4%
Std 1 - 0.128nM	15142	3%
Std 2 - 0.64nM	14968	3%
Std 3 - 3.2nM	12596	5%
Std 4 - 16nM	8071	2%
Std 5 - 80nM	2322	3%
Std 6 - 400nM	436	4%
Std 7 - 2000nM	418	4%



ANALYTICAL CHARACTERISTICS

HTRF MI-1061 Red Ligand Kd	10.9nM
HTRF MI-1061 Red Ligand concentration	2nM
HTRF MDM2 Binding Kit - Standard IC50	16.6nM
HTRF MDM2 Binding Kit - Standard Ki	14nM

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