6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

MDM2 TR-FRET Assay Kit

Catalog #79773
Size: 384 reactions

DESCRIPTION:

Human Casitas B-lineage lymphoma proto-oncogene b (MDM2) is an E3 ubiquitin-protein ligase that functions as a negative regulator of T-cell activation. It is a potential drug target in cancer immunotherapy. The *MDM2 TR-FRET Assay Kit* is designed to measure MDM2 auto-ubiquitination activity in a homogeneous 384 reaction format. It utilizes biotin-labeled ubiquitin and a terbium-labeled anti-GST antibody to complete the TR-FRET pairing. This FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications.

COMPONENTS:

Catalog #	Component	Amount	Storage	
80301	UBE1 (E1)	25 µg	-80°C	***Avoid freeze/ thaw cycles!***
80314	UBCH5b (E2)	200 µg	-80°C	
80751	Human MDM2 (E3), GST-tag	10 µg	-80°C	
	Biotin-Ubiquitin	400 µl	-80°C	
	ATP (400 µM)	150 µl	-80°C	
	CBL assay buffer	2 x 10 ml	-80°C	
	Tb-labeled donor	10 µl	-20°C	
	Dye-labeled acceptor	10 µl	-20°C	
	White, nonbinding Corning, low volume microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: At least 6 months from date of receipt when stored as directed.

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REFERENCE: 1. Wienken, M. *et al., J. Mol. Cell Biol.* 2017; **9(1):** 74-80.
2. Shaikh, M.F. *et al., Ann. Clin. Lab. Sci*. 2016; **46(6):** 627-34.

ASSAY PROTOCOL:

All samples and controls should be tested in triplicates.

1) Thaw **UBE1, UBCH5b, MDM2, Biotin-Ubiquitin, CBL assay buffer**, and **ATP** on ice. Aliquot each protein, assay buffer, and ATP into single-use aliquots and immediately store at -80°C. *Note: **UBE1, UBCH5b, MDM2, Biotin-Ub**, and **CBL assay buffer** are sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*

2) Carefully calculate the amount of proteins needed. Prepare appropriate amounts of diluted proteins as needed:

   Dilute the **UBE1** in **CBL assay buffer** at 59 ng/µl**;**
   Dilute the **UBCH5b** in **CBL assay buffer** at 180 ng/µl**;**
   Dilute the **MDM2** in **CBL assay buffer** at 7 ng/µl**;**

   Keep the diluted reagents on ice until use.

3) Prepare the master mixture using diluted reagents: N wells × (1 µl **Biotin-Ub** + 1 µl **UBE1** + 1 µl **UBCH5b** + 2.5 µl **MDM2**). Add 5.5 µl of master mixture to each well designated for the "Substrate Control", "Positive Control", "Test Sample". For the wells labeled "blank", add 1 µl **Biotin-Ub** + 1 µl **UBE1** + 1 µl **UBCH5b** + 2.5 µl **CBL assay buffer.**

	Test Sample	**Substrate Control**	**Positive Control**	**Blank**
Biotin-Ub	1 µl	1 µl	1 µl	1 µl
UBE1	1 µl	1 µl	1 µl	1 µl
UBCH5b	1 µl	1 µl	1 µl	1 µl
MDM2	2.5 µl	2.5 µl	2.5 µl	–
Test Inhibitor/Activator	2 µl	–	–	–
Inhibitor buffer* (no inhibitor)	–	2 µl	2 µl	2 µl
CBL assay buffer	–	2.5 µl	–	2.5 µl
ATP (4 µM)	2.5 µl	–	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

*Typically, Inhibitor buffer represents assay buffer with proper concentration of DMSO.

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4) Prepare the inhibitor solution.

   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound in DMSO than the highest concentration you want to test in the assay. Then make a 20-fold dilution in 1X assay buffer (at this step the compound concentration is 5-fold higher than the final concentration). If you want to run an IC50 or test lower concentrations of the compound, make a series of further dilutions using 1X assay buffer containing 5% DMSO, so the final concentration of DMSO will be 1% in all samples.

   If the inhibitor compound is dissolved in water, make a solution of the compound in 1X assay buffer that is 5-fold higher than the final assay concentration.

5) Add 2 µl of inhibitor solution of each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 2 µl of the same solution without inhibitor (Inhibitor buffer).

6) Dilute the **ATP** stock (400 µM) 100-fold using **CBL assay buffer** to 4 µM. Initiate the reaction by adding 2.5 µl of diluted **ATP** to the wells labeled "Positive Control", "Test Inhibitor", and "Blank". Add 2.5 µl of **CBL assay buffer** to the well designated "Substrate Control". Incubate the reaction at 30°C for three hours. Cover the plate with a plate sealer if necessary.

7) Thaw **CBL assay buffer** on ice. Dilute **Tb-labeled donor** (1:400) and **Dye-labeled acceptor** (1:400) in one step using **CBL assay buffer**. Add 10 µl **diluted donor/acceptor mixture** into each well. Incubate at room temperature for one hour.

8) Read the fluorescent intensity in a microtiter-plate reader capable of measuring TR-FRET. "Blank" value is subtracted from all other values.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	340±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

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CALCULATING RESULTS:

Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

When percentage activity is calculated, the FRET value from the negative control (Blank or Substrate Control) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\% \text{ Activity} = \frac{FRET_s - FRET_{neg}}{FRET_p - FRET_{neg}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{neg}$ = negative control FRET, and $FRET_P$ = Positive control FRET.

Example of Assay Results:

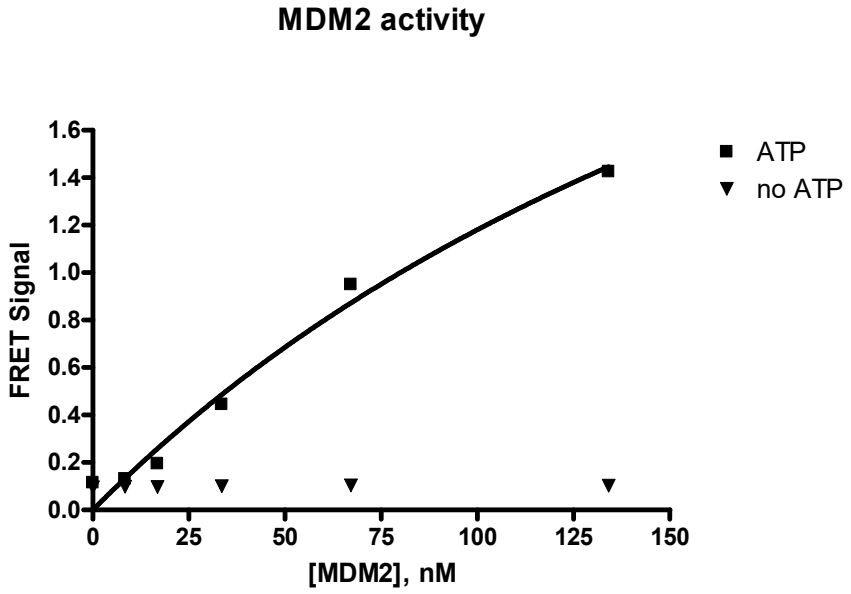

Figure 1: Titration of MDM2 activity using the *MDM2 TR-FRET Assay Kit*, BPS Bioscience #79773. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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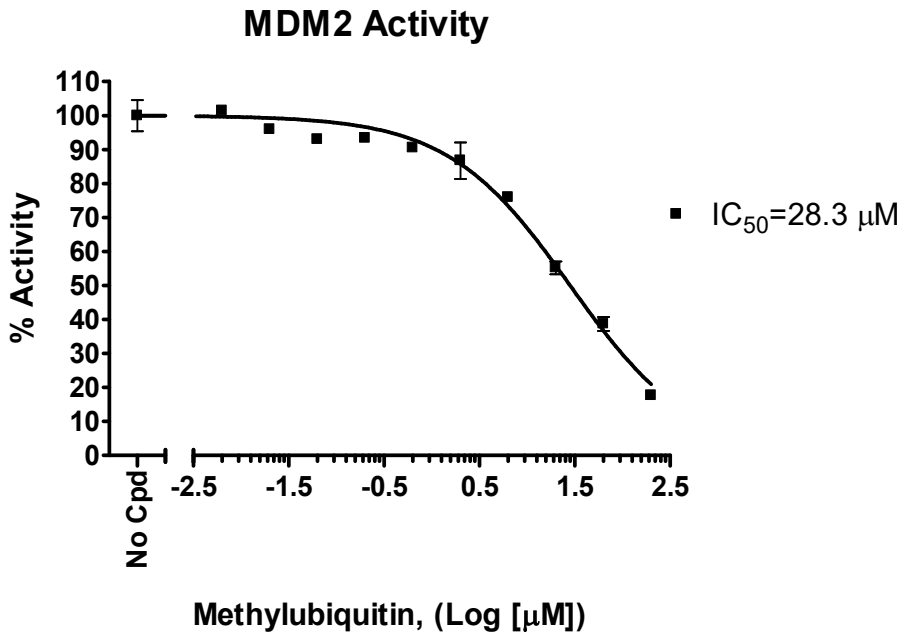

Figure 2: Inhibition of MDM2 Assay FRET signal by Methylated Ubiquitin, measured using the *MDM2 TR-FRET Assay Kit*, BPS Bioscience #79773. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS

Product Name	**Catalog #**	**Size**
MDM2, GST-Tag (Human)	#80751	20 µg
UBE1 (UBA1), FLAG-tag	#80301	100 µg
UBCH5b	#80314	100 µg
CBL-B, GST-Tag (Human)	#80415	100 µg
CBL-B, His-Avi-Tag	#80414	100 µg
CBL-B, Biotin-labeled (Human)	#80412	50 µg
CBL-B (Y363F), Biotin-labeled (Human)	#80413	50 µg

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