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Data Sheet

UBCH5c TR-FRET Assay Kit

Catalog # 79901
Size: 384 reactions

DESCRIPTION:

UBCH5c (UBE2D3) is an E2 conjugating ubiquitin protein that functions in the ubiquitination of the tumor-suppressor protein p53. It is a promising drug target in cancer immunotherapy. The *UBCH5c TR-FRET Assay Kit* is designed to measure ubiquitination activity in a homogeneous 384 reaction format. It utilizes biotin-labeled Ubiquitin and a terbium-labeled antibody recognizing the His-tagged UBCH5c protein 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!***
80313	UBCH5c (E2)	5 µg	-80°C	
	Biotin-Ubiquitin	400 µl	-80°C	
	ATP (10 mM)	400 µl	-80°C	
	CBL assay buffer	2 x 10 ml	-80°C	
30017	Anti-His Tb-labeled donor	10 µl	-20°C	
	Dye-labeled acceptor	10 µl	-20°C	
	Methyl-ubiquitin (1 mM in 50 mM Tris, pH 7.4)	20 µl	-80 °C	
	White Corning 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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REFERENCES: 1. Saville, M.K., *et al., J. Biol. Chem*. 2004; **279(40):** 42169-81.
2. Kim, J.-H., *et al., BMB Rep.* 2015; **48(1):** 25-29.

ASSAY PROTOCOL:

All samples and controls should be tested in triplicates.

1) Thaw **UBE1, UBCH5c, Biotin-Ubiquitin, CBL assay buffer**, and **ATP** on ice. Aliquot each protein, **CBL assay buffer**, and **ATP** into single-use aliquots and store at -80°C immediately. *Note: **UBE1, UBCH5c, Biotin-Ub, CBL assay buffer**, and **ATP** 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; dilute only the amount required for the assay. Do not store diluted proteins

   Dilute the **UBE1** in **CBL assay buffer** at 40 ng/µl
   Dilute the **UBCH5c** in **CBL assay buffer** at 8 ng/µl
   Keep the diluted reagents on ice until use.

3) Prepare the master mixture using diluted reagents: N wells × (1 µl **Biotin-Ub** + 1.5 µl diluted **UBE1** + 1.5 µl diluted **UBCH5c**). Add 4 µl of master mixture to each well designated for the "Substrate Control," "Positive Control," "Test Inhibitor." For the wells labeled "Blank," add 1 µl **Biotin-Ub**+ 1.5 µl diluted **UBE1** + 4.5 µl **CBL assay buffer**.

4) Dilute your test inhibitor (100x in DMSO) 1:20 in distilled water. Add 2 µl of diluted test inhibitor solution to each well designated "Test Inhibitor." For the "Positive Control," "Substrate Control," and "Blank," add 2 µl of the same solution without the test inhibitor (inhibitor buffer), in this instance, 5% DMSO in water. Final DMSO concentration in the assay should be ≤1%.

   Note: To make an IC50 using the methyl-ubiquitin, prepare serial dilutions in the same inhibitor buffer as your test inhibitor (typically 5% DMSO in water)

5) Add 4 µl of assay buffer to the well designated "Substrate Control." Add 3 µl of assay buffer to the wells designated "Positive Control," and "Test Inhibitor."

6) Initiate the reaction by adding 1 µl of **ATP (10 mM)** to the wells labeled "Positive Control," "Test Inhibitor," and "Blank." Incubate the reaction at 30°C for four hours. Cover the plate with a plate sealer if necessary.

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	Blank	Substrate Control	Positive Control	Test Inhibitor
Biotin-Ub	1 µl	1 µl	1 µl	1 µl
UBE1 (40 ng/µl)	1.5 µl	1.5 µl	1.5 µl	1.5 µl
UBCH5c (8 ng/µl)	–	1.5 µl	1.5 µl	1.5 µl
Test Inhibitor/Activator	–	–	–	2 µl
5% DMSO in water (Inhibitor buffer)	2 µl	2 µl	2 µl	–
CBL assay buffer	4.5 µl	4 µl	3 µl	3 µl
ATP (10 mM)	1 µl	–	1 µl	1 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

7) Dilute **Tb-labeled donor** (1:400) and **Dye-labeled acceptor** (1:400) in one step using CBL Assay Buffer. Prepare only the amount required for the assay. Add 10 µl of 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:

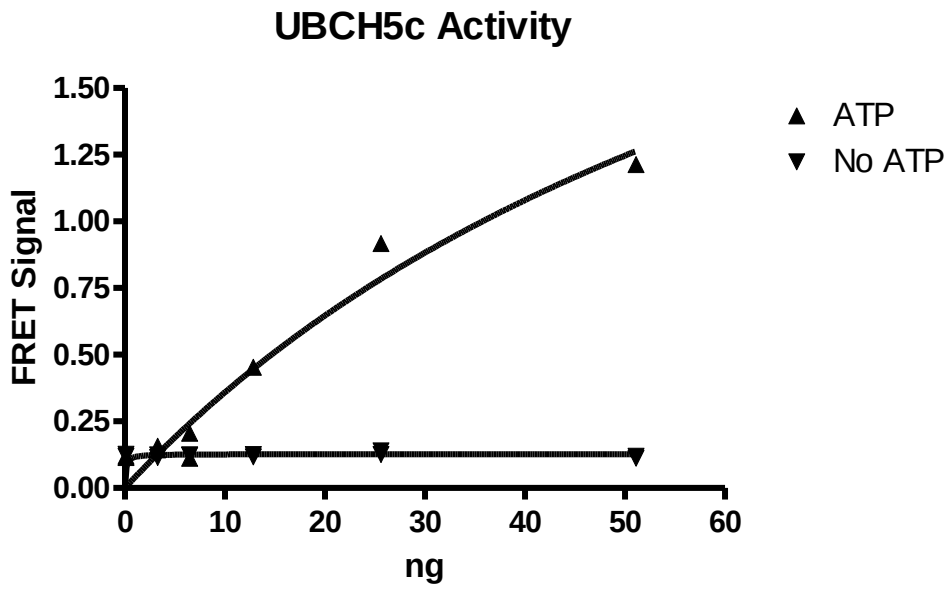

Figure 1: Titration of UBCH5c activity using the *UCBH5c TR-FRET Assay Kit*, BPS Bioscience #79901. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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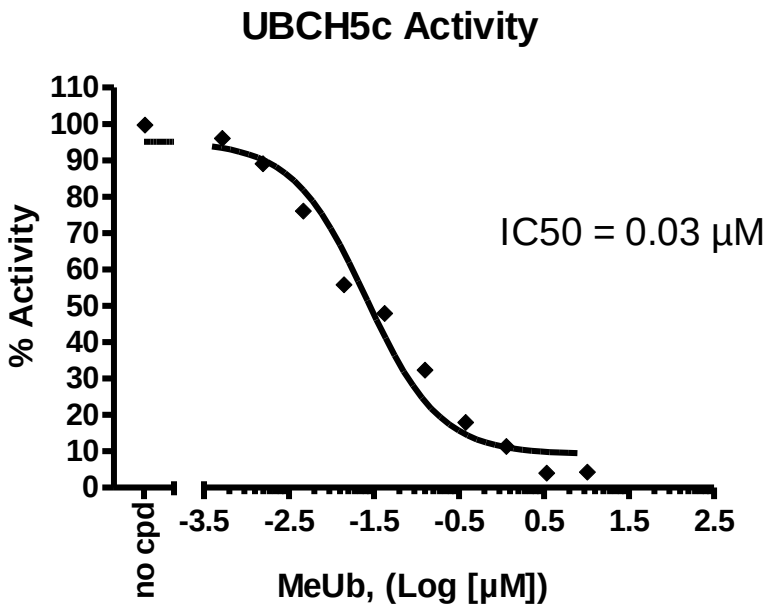

Figure 2: Inhibition of UCBH5c Assay FRET signal by Methylated Ubiquitin, measured using the *UCBH5c TR-FRET Assay Kit*, BPS Bioscience #79901. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS

Product Name	**Catalog #**	**Size**
UBE1 (UBA1), FLAG-tag	#80301	100 µg
Ubiquitin, Biotin Labeled	#11236	50 µg
UbcH5a, His-Tag (Human)	#80315	100 µg
UbcH5b, His-Tag (Human)	#80314	100 µg
UbcH5c, His-Tag (Human)	#80313	100 µg
UBA6 (UBE1L2), FLAG-tag	#80303	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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