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Data Sheet

UBCH13 TR-FRET Assay Kit

Catalog #79741
Size: 384 reactions

DESCRIPTION:

UBCH13 is a noncanonical ubiquitin conjugating enzyme (E2) that has been implicated in a variety of cellular signaling processes due to its ability to catalyze the formation of lysine 63-linked polyubiquitin chains on various substrates. In particular, UBCH13 is required for signaling by a variety of receptors important in immune regulation, making it a candidate target for inflammatory diseases. UBCH13 is also critical for double-strand DNA repair and thus a potential radiosensitizer and chemosensitizer target for oncology.
The *UBCH13 TR-FRET Assay Kit* is designed to measure UBCH13 ubiquitination activity in a homogeneous 384 reaction format. It utilizes biotin-labeled ubiquitin and a terbium-labeled anti-His 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:

Cat. #	Component	Amount	Storage	
80301	UBE1 (E1)	25 µg	-80°C	***Avoid freeze/ thaw cycles!***
80323	Human UBCH13 (UBE2N), His-tag	10 µg	-80°C	
	Biotin-Ubiquitin	450 µl	-80°C	
	ATP (400 µM)	150 µl	-80°C	
	UBCH assay buffer	2x 5 ml	-80°C	
	Tb-labeled donor	2x 10 µl	-20°C	
	Dye-labeled acceptor	2x 10 µl	-20°C	
	Detection buffer	5 ml	-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

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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.

REFERENCE: Madiraju, C., *et al., J. Biomol. Screen.* 2012;**17(2):**163-76.

ASSAY PROTOCOL:
All samples and controls should be tested in triplicates.

1) Thaw UBE1, UBCH13, Biotin-Ubiquitin, UBCH assay buffer, and ATP on ice. Briefly spin each tube to recover the full contents of the tube. Aliquot each protein, assay buffer, and ATP into single-use aliquots and stored at -80°C immediately. *Note: UBE1, UBCH13, Biotin-Ub, 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 as needed:

   Dilute the UBE1 in UBCH assay buffer at 30 ng/µl**.**
   Dilute the UBCH13 in UBCH assay buffer at 9 ng/µl**.**
   Keep the diluted reagents on ice until use.

3) Prepare the master mixture using diluted reagents: N wells × (1 µl Biotin-Ub + 2 µl diluted UBE1 + 2.5 µl diluted UBCH13). Add 5.5 µl of master mixture to each well designated for the “Substrate Control”, “Positive Control”, “Test Inhibitor”. For the wells labeled as “blank”, add 1 µl Biotin-Ub+ 2 µl UBE1 + 2.5 µl UBCH assay buffer.

4) Add 2 µl of inhibitor solution to each well designated “Test Inhibitor”. For the “Positive Control”, “Substrate Control” and “Blank”, add 2 µl of the same solution without inhibitor (inhibitor buffer*). *Note: Keep DMSO concentration of the Test Inhibitor at ≤5%, as final DMSO concentration in the reaction should be ≤1%.*

5) Add 2.5 µl of assay buffer to the wells designated “Substrate Control”.

6) Dilute the ATP stock (400 µM) 100-fold using 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”. Incubate the reaction at 30°C for two hours. Cover the plate with a plate sealer if necessary to prevent evaporation.

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	Blank	Substrate Control	Positive Control	Test Inhibitor
Biotin-Ub	1 µl	1 µl	1 µl	1 µl
UBE1	2 µl	2 µl	2 µl	2 µl
UBCH13	–	2.5 µl	2.5 µl	2.5 µl
Test Inhibitor/Activator	–	–	–	2 µl
Inhibitor buffer* (no inhibitor)	2 µl	2 µl	2 µl	–
UBCH13 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**

*Inhibitor buffer represents assay buffer with the proper concentration of DMSO to mimic the DMSO concentration in the inhibitor dilutions.

7) Thaw Detection Buffer on ice. Dilute Tb-labeled donor (1:200) and Dye-labeled acceptor (1:200) in one step using Detection Buffer. For example, add 20 µl Tb-labeled donor, 20 µl Dye-labeled acceptor, and 3.96 ml Detection buffer. Dilute only enough Tb-labeled donor and Dye-labeled acceptor as required for the assay. 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.

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

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

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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:

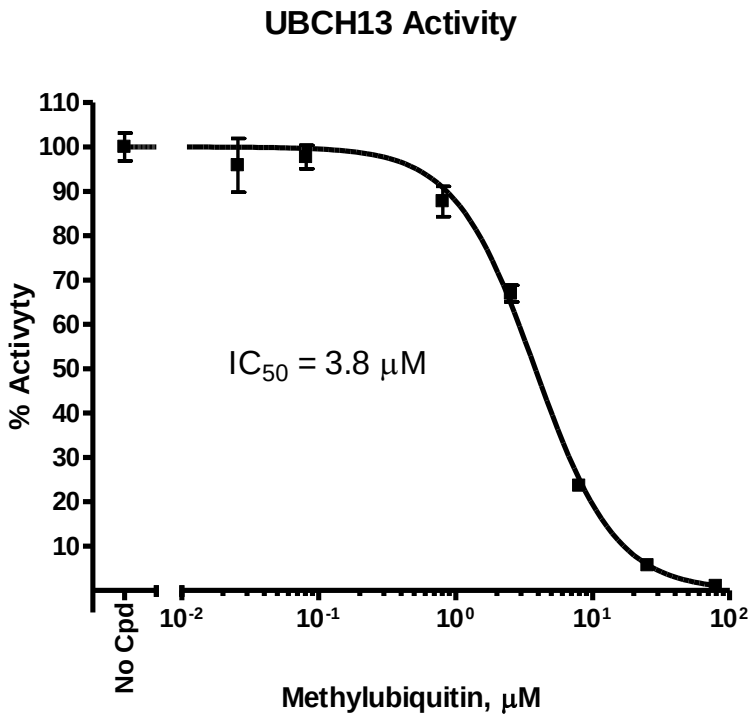

Figure 1: Inhibition of UBCH13 Assay FRET signal by Methylated Ubiquitin, measured using the *UBCH13 TR-FRET Assay Kit*, BPS Bioscience #79741. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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RELATED PRODUCTS

Product Name	Catalog #	Size
UbcH13 (UBE2N), His-tag	#80323	100 µg
Ubiquitin, His-Tag	#79293	2 mg
Ubiquitin, His-Avi-Tag, Biotin Labeled	#11236	50 µg
UbcH5a (UBE2D1), His-tag	#80315	100 µg
UbcH5b, His-tag	#80314	100 µg
UbcH6 (UBE2E1), His-tag	#80316	100 µg
UbcH7, His-tag (E. coli)	#80317	100 µg
USP7, His-FLAG-tags	#80593	100 µg
USP7 Inhibitor Screening Assay Kit	#79256	96 rxns.
UBE1 (UBA1), FLAG-tag	#80301	100 µg
UEV1A (UBE2V1), FLAG-tag	#80312	100 µg