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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Ubi-Trans™ UBA6-UbcH5b TR-FRET Assay Kit is a homogeneous, sensitive TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer) assay designed to measure UBA6 (ubiquitin-like modifier activating enzyme 6)-dependent transfer of Ub (ubiquitin) to UbcH5b (Ubiquitin-conjugating enzyme E2 D2) E2 enzyme. It utilizes a Terbium (Tb)-labeled donor and dye-labeled streptavidin acceptor to complete the TR-FRET pairing. The kit comes in a convenient 384-well format and contains enough purified UbcH5b, purified UBA6, Biotin-Ubiquitin, anti-His Tb (terbium)-labeled donor, dye-labeled streptavidin acceptor, and assay buffer for 400 reactions.

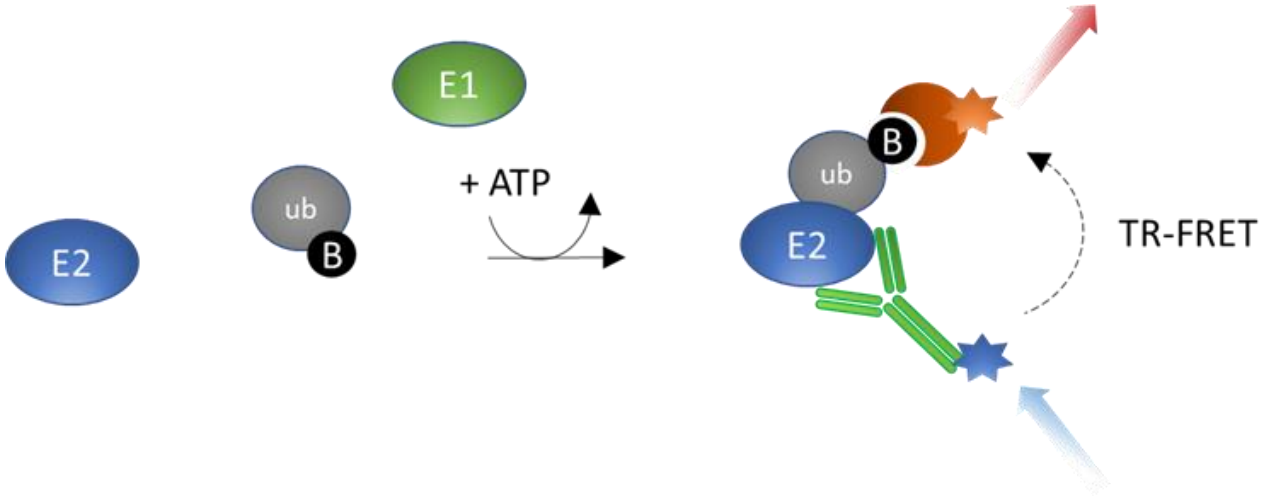

Figure 1: Ubi-Trans™ UBA6-UbcH5b TR-FRET Assay Kit schematic.

In the presence of ATP, UBE6 (E1) transfers biotin-conjugated ubiquitin to the substrate UbcH5b (E2). The Tb-labeled anti-His antibody binds to the His-tagged E2 conjugating protein, while the dye-labeled streptavidin acceptor binds to Biotin-Ubiquitin. The complex forms when ubiquitin is transferred to the E2 enzyme by E1. In the absence of ubiquitination, the complex does not form and energy transfer does not occur. When E2 is ubiquitinated the complex is formed, TR-FRET pairing is complete, and an increase in acceptor emission is observed. Thus, TR-FRET signal is proportional to UBE6 activity.

Background

Ubiquitination is a multistep process that involves E1 (ubiquitin-activating enzymes), E2 (ubiquitin-conjugating enzymes) and E3 (ubiquitin ligase) enzymes. In the first step, an E1-ubiquitin thioester bond is formed between the C-terminal glycine carboxyl group of ubiquitin and the cysteine in the active site of the E1 enzyme in an ATP-dependent reaction. Next, the E1 enzyme transfers the activated ubiquitin to the cysteine residue of the E2 enzyme to form an E2-ubiquitin thioester-linked intermediate by transesterification. Finally, the E2 enzyme transfers ubiquitin to the substrate protein with the help of an E3 ligase. UBA6 (ubiquitin-like modifier activating enzyme 6) is an E1 enzyme that activates ubiquitin but also has the unique feature of being able to act on FAT10 (also known as ubiquitin D or human leukocyte antigen-F adjacent transcript 10). UBA6 is present in most tissues at very low levels, however it is upregulated during dendritic cell maturation and hyperthermic stress. UBA6 can transfer ubiquitin to UbcH5b (also known as Ubiquitin-conjugating Enzyme E2 D2, UBE2D2). UbcH5b is overexpressed in certain cancer types. It ubiquitinates the tumor-suppressor proteins p53 and p62, promoting their degradation. The UbcH5b-p62 complex confers therapeutic resistance in triple negative breast cancer cells. UbcH5b is also involved in endocytosis-mediated degradation of MHC (major histocompatibility complex) class I molecules, and overexpression has been found in inflammatory bowel disease. Inhibitors of ubiquitination of UbcH5b by UBE6 may prove beneficial in cancer and auto-immune disease treatment.

Applications

Screen molecules that inhibit UBA6-dependent transfer of Ub to UbcH5b in drug discovery high-throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
80303	UBA6 (UBE1L2), FLAG-Tag*	4.8 µg	-80°C
80314	UbcH5b, His-Tag (Human)*	5 µg	-80°C
	Biotin-Ubiquitin	80 µl	-80°C
	4 mM ATP	1 ml	-80°C
78856	U2 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
79969	White, nonbinding, low volume microtiter plate		Room Temp

** The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

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

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The Ubi-Trans™ UBA6-UbcH5b TR-FRET Assay Kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in triplicate.
- The assay should include "Blank", "Positive Control", "Negative Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using TAK243 as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1) Thaw **UBA6**, **UbcH5b**, **U2 Assay Buffer**, **Biotin-Ubiquitin**, and **ATP** on ice. Briefly spin the tubes to recover their full content.

2) Dilute **UBA6** in U2 Assay Buffer to 8 ng/µl (1.5 µl/well).

3) Dilute **UbcH5b** in U2 Assay Buffer to 8 ng/µl (1.5 µl/well).

4) Dilute **Biotin-Ubiquitin** 5-fold with U2 Assay Buffer (1 µl/well).

5) Prepare a **Master Mix** (5.5 µl/well): N wells x (1.5 µl of diluted UBA6 + 1.5 µl of diluted UbcH5b + 1 µl of diluted Biotin-Ubiquitin + 1.5 µl of U2 Assay Buffer).

6) Add 5.5 µl of **Master Mix** to the "Positive Control", "Negative Control" and "Test Inhibitor" wells.

7) Add 5.5 µl of U2 Assay Buffer to the "Blank" wells.

8) Prepare the Test Inhibitor (2 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 20 µl.

   8.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in U2 Assay Buffer, 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use U2 Assay Buffer (Diluent Solution).

   OR

   8.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute 10-fold in U2 Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in U2 Assay Buffer to keep the concentration of DMSO constant.

   For controls prepare 10% DMSO in U2 Assay Buffer (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

9) Add 2 µl of inhibitor solution to each well designated "Test Inhibitor".

10) Add 2 µl of the Diluent Solution to the "Positive Control", "Negative Control" and "Blank" wells.

Note: Consider preincubating enzymes with the inhibitor for 30 minutes at Room Temperature (RT) prior to adding the substrate.

11) Dilute Anti-His Tb-Labeled Donor and Dye-Labeled Acceptor 400-fold, together, in U2 Assay Buffer (10 µl of mix/well). This makes the **Donor/Acceptor Mix**.

12) Add 10 µl of Donor/Acceptor Mix to each well.

13) Initiate the reaction by adding 2.5 µl of **4 mM ATP** to the "Blank", "Test Inhibitor", and "Positive Control" wells.

14) Add 2.5 µl U2 Assay Buffer to the "Negative Control" wells.

	Blank	**Test Sample**	**Positive Control**	**Negative Control**
Master Mix	-	5.5 µl	5.5 µl	5.5 µl
U2 Assay Buffer	5.5 µl	-	-	2.5 µl
Test Inhibitor	-	2 µl	-	-
Diluent solution	2 µl	–	2 µl	2 µl
Donor/Acceptor Mix	10 µl	10 µl	10 µl	10 µl
4 mM ATP	2.5 µl	2.5 µl	2.5 µl	-
Total	**20 µl**	**20 µl**	**20 µl**	**20 µl**

15) Protect from light and incubate the reaction at RT for 30 minutes or perform kinetic analysis for up to 1 hour.

16) Read the fluorescent intensity in a microtiter-plate reader capable of measuring TR-FRET.

17) The "Blank" value should be subtracted from all other values.

Instrument Settings

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).

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

Calculate the FRET value by using the following formula:

$$FRET = \frac{S_{665} - \left(\frac{Tb_{665}}{Tb_{620}} \times S_{620}\right)}{S_{620}} \times 1000$$

S_{665} = Sample value measured at 665 nm, S_{620} = Sample value measured at 620 nm, Tb_{665} = Tb only or Blank value measured at 665 nm, Tb_{520} = Tb only or Blank value measured at 520 nm.

The FRET value calculated for the negative control should be subtracted from all other measurements and can be set as 0%. The FRET value from the "Positive Control" can be set as 100% activity.

$$\%\ Activity = \frac{FRET_S - FRET_{neg}}{FRET_P - FRET_{neg}} \times 100\%$$

$FRET_S$ =FRET value for samples of Test Inhibitor, $FRET_{Sub}$ = FRET value for the Substrate Control, and $FRET_p$ = FRET value for the Positive Control (no inhibitor).

Example Results

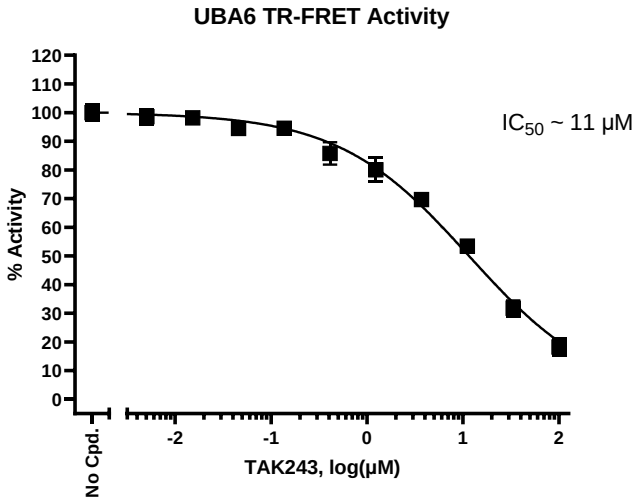

Figure 2: UBA6-dependent transfer of Ub to UbcH5b activity is inhibited by TAK243.
Transfer of Ub to UbcH5b was measured in the presence of increasing concentrations of TAK243 inhibitor (Selleckchem #S8341). Results are expressed as percentage of activity relative to positive control (measured in the absence of inhibitor and set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For further questions, email support@bpsbioscience.com

References
Saville M.K., *et al.,* 2004 *J. Biol. Chem.*279(40): 42169-81.
Kim J.H., *et al.,* 2015 *BMB Rep.* 48(1): 25-29.
Truongvan N., *et al.*, 2022 *Nature Communications* 12:4789.

Related Products

Products	*Catalog #*	*Size*
ChoosE3-Freedom™ Intrachain TR-FRET Assay Kit	78560	384 reactions
ChoosE2-Opti™ Intrachain TR-FRET Assay Kit	78561	384 reactions
UBE1 Inhibitor Screening Assay Kit	79957	96 reactions
UBE1, GST-Tag Recombinant	100402	100 µg
UBE1-UcbH5b TR-FRET Assay Kit	82215	384 reactions

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