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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The TNF receptor associated factor 6 (TRAF6) TR-FRET Assay Kit is a sensitive TR-FRET Assay Kit, designed to measure TRAF6 (amino acids 51-159) auto-ubiquitination activity in a homogeneous 384 reaction format. This assay measures mono- or poly-ubiquitination. As a homogeneous assay, it requires no time-consuming washing steps and is especially suitable for high throughput screening (HTS) applications as well as real-time analyses. The kit contains enough purified UBE1 (E1), UBCH5b (E2), E3 ligase TRAF6 (amino acids 51-159, GST-tag), Biotin-Ubiquitin, labelled donor and acceptor, ATP, and U2 Assay Buffer for 384 reactions. Read out requires a fluorescent plate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET).

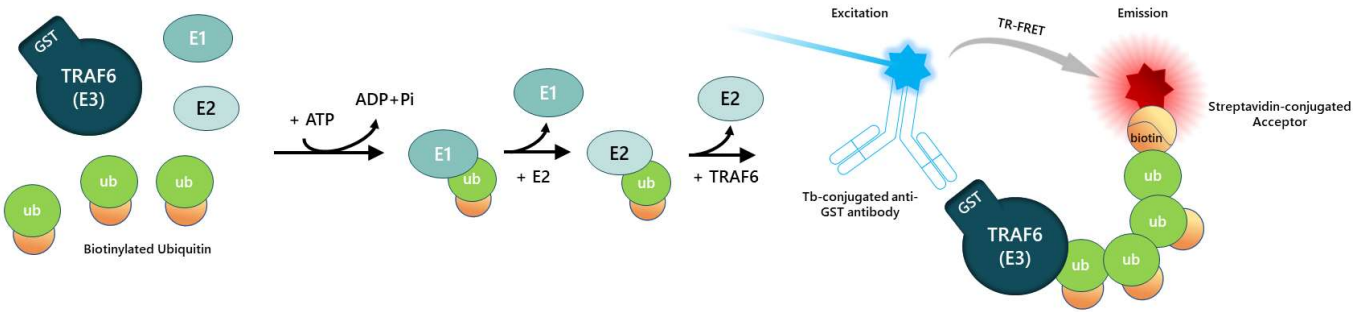

Figure 1. TRAF6 TR-FRET Assay Kit schematic.

The assay takes advantage of biotinylated ubiquitin, which is linked to TRAF6 during auto-ubiquitination. The streptavidin-conjugated acceptor binds to ubiquitin-biotin while a Terbium-conjugated anti-GST antibody donor binds to GST-tagged TRAF6, allowing TR-FRET pairing. The resulting TR-FRET signal is directly proportional to TRAF6 mono or poly-ubiquitination.

Background

Covalent conjugation of proteins to ubiquitin (Ub) is one of the major post-translational modifications that regulates protein stability, function, and localization. Ubiquitination is the concerted action of three enzymes: a Ub-activating enzyme (E1), a Ub-conjugating enzyme (E2), and a Ub ligase (E3). The specificity and efficiency of ubiquitination are largely determined by the E3 enzyme, which directs the last step of the Ub-conjugating cascade by binding to both an E2~Ub conjugate and a substrate protein. This step ensures the transfer of Ub from E2~Ub to the substrate, leading to its mono- or poly-ubiquitination. TRAF6 (TNF receptor-associated factor 6) is an E3 ligase, involved in inflammation and innate immunity. Inhibitors of TRAF6 activity impact the NF-κB pathway and can prove beneficial for the treatment of auto-immune and chronic inflammatory disorders, such as psoriasis and rheumatoid arthritis.

Supplied Materials

Catalog #	Name	Amount	Storage
80301	UBE1 (UBA1), Flag-Tag*	50 µg	-80°C
80314	UBCH5b, His-Tag (Human)*	60 µg	-80°C
101597	TRAF6, GST-Tag*	8 µg	-80°C
11236	Ubiquitin, His-Avi Tag, Biotin-Labeled	2 x 12 µg	-80°C
	Tb-labeled donor	2 x 10 µl	-20°C
	Dye-labeled acceptor	2 x 10 µl	-20°C
	ATP (4 mM)	2 x 1 ml	-80°C
	U2 Assay Buffer	2 x 10 ml	-80°C
	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

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed. ***Avoid multiple freeze/thaw cycles!***

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 TRAF6 intrachain TR-FRET Assay Kit is compatible with up to 1% final DMSO concentration.
- TRAF6, GST-Tag (BPS Bioscience #101597) is comprised of amino acids 51-159. Inhibitors targeting TRAF6 regions outside of the 51-159 amino acid region cannot be assessed using this assay kit.

Assay Protocol

- All samples and controls should be performed in triplicates.
- The assay should include a “Blank”, a “Positive Control”, and a “Negative Control”.
- If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots, depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.

1) Thaw **UBE1**, **UBCH5b**, **TRAF6, Biotin-Ubiquitin**, **U2 Assay Buffer**, and **ATP** on ice. Briefly spin the tubes to recover their full content.

2) Dilute **UBE1** in U2 Assay Buffer to 48 ng/µl (final concentration in the reaction will be 40 nM) (2 µl/well).

3) Dilute **UBCH5b** in U2 Assay Buffer to 72 ng/µl (final concentration in the reaction will be 400 nM) (2 µl/well).

4) Dilute **TRAF6** in U2 Assay Buffer to 4 ng/µl (final concentration in the reaction will be 25 nM) (5 µl/well).

5) Dilute **Biotin-Ubiquitin** in U2 Assay Buffer to 30 ng/µl (final concentration in the reaction will be 250 nM) (2 µl/well).

   Note: UBE1, UBCH5b, TRAF6, Biotin-Ubiquitin, and U2 Assay Buffer are sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles. Keep all diluted proteins on ice until use. Do not freeze and re-use diluted proteins.

6) Prepare a Master Mix: N wells x (2 µl of diluted Biotin-Ubiquitin + 2 µl of diluted UBE1 + 2 µl of diluted UBCH5b + 5 µl of diluted TRAF6).

7) Add 11 µl of Master Mix to "Positive Control", "Negative Control", and "Test Inhibitor" wells.

8) Prepare a Deficient Master Mix (**without TRAF6**) for the "Blank" wells: N wells x (2 µl of diluted Biotin-Ubiquitin + 2 µl of diluted UBE1 + 2 µl of diluted UBCH5b + 5 µl of U2 Assay Buffer).

9) Add 11 µl of Deficient Master Mix to the "Blank" wells.

10) Prepare the Test Inhibitor (4 µl/well): for a titration, prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 20 µl.

    10.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in the U2 Assay Buffer, 5-fold more concentrated than the desired final concentrations.
    For the positive and negative controls, use U2 Assay Buffer (Diluent Solution).

    10.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 20-fold in U2 Assay Buffer to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.
    Prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations using 5% DMSO in U2 Assay Buffer to keep the concentration of DMSO constant.
    For positive and negative controls, prepare 5% DMSO in U2 Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).
    The final concentration of DMSO should not exceed 1%.

11) Add 4 µl of test inhibitor dilutions to the "Test Inhibitor" wells.

12) Add 4 µl of the Diluent Solution to the "Positive Control", "Negative Control" and "Blank" wells.

13) Initiate the reaction by adding 5 µl of **ATP** to the "Positive Control" and "Test Inhibitor" and "Blank" wells.

14) Add 5 µl of **U2 Assay Buffer** top the "Negative Control" wells.

15) Cover the plate with a lid and incubate the reaction at Room Temperature for one hour.

	Inhibitor Test	**Negative Control**	**Positive Control**	**Blank**
Master Mix	11 µl	11 µl	11 µl	-
Deficient Master Mix	-	-	-	11 µl
Test Inhibitor	4 µl	–	–	-
Diluent Solution	–	4 µl	4 µl	4 µl
U2 Assay Buffer	–	5 µl	–	-
ATP (4 mM)	5 µl	–	5 µl	5 µl
Total	**20 µl**	**20 µl**	**20 µl**	**20 µl**

16) Prepare a solution containing both Tb-labeled donor and Dye-labeled acceptor, each diluted at 1:400 with U2 Assay Buffer.

17) Add 20 µl of the mix into each well.

18) Incubate at Room Temperature for one to three hours.

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

20) "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

CALCULATING RESULTS

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

To calculate the percentage activity the FRET value from the "Blank" should be subtracted from all other values, and the FRET value from the positive control can be set as one hundred percent activity.

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

Where FRETs = Sample FRET, $FRET_{blank}$ = Blank FRET, and $FRET_P$ = Positive Control FRET.

Example Results

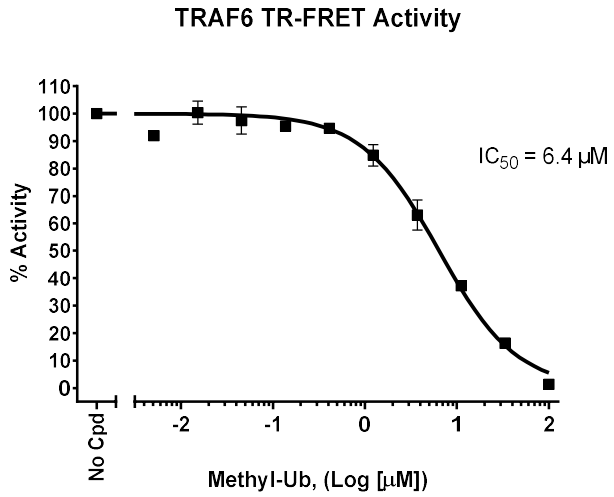

Figure 2: Inhibition of TRAF6 auto-ubiquitination by Methyl-Ub.
TRAF6 auto-ubiquitination was measured in the presence of increasing concentrations of Methylated Ubiquitin. Results are expressed as percent of control activity (measured in the absence of inhibitor and set at 100%).

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 all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
TRAF6- GST-Tag Recombinant	101597	2 µg/50 µg
TRAF6 (D57K), GST-Tag Recombinant	101598	2 µg/50 µg
CBL-B TR-FRET Assay Kit	79575	384 reactions
C-CBL TR-FRET Assay Kit	79786	384 reactions