SZABO SCANDIC

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# Produktinformation

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

Weitere Information auf den folgenden Seiten!
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 WWP1 intrachain TR-FRET Assay Kit is a sensitive high-throughput screening (HTS) TR-FRET Assay Kit, designed to measure WWP1 auto-ubiquitination activity in a homogeneous 384 reaction format. It utilizes a Europium cryptate-labeled Ubiquitin (donor) as well as a Cy5-labeled Ubiquitin (acceptor) to complete the TR-FRET pairing. Since both the TR-FRET donor and acceptor are incorporated into poly-ubiquitin chains formed on WWP1, this FRET-based assay requires no time-consuming washing steps, making it especially suitable for HTS applications as well as real-time kinetic analyses of polyubiquitination.

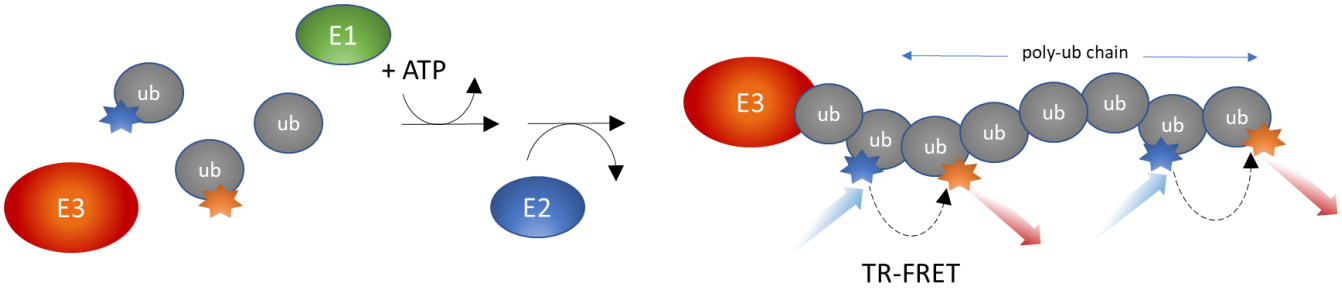


*Figure 1. WWP1 intrachain TR-FRET Assay Kit schematic*

**Background**

Covalent conjugation 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.

WW Domain Containing E3 Ubiquitin Protein Ligase 1 (WWP1) is a HECT-type E3 Ub ligase belonging to the NEDD4 family of E3 ligases. WWP1 interacts with and ubiquitinates many substrates, including transcription factor ΔNp63 as well as phosphatase and tensin homolog (PTEN), and regulates the degradation of sodium channels and membrane receptors. Genetic mutations or regulatory defects involving WWP1 are associated with neurological disorders and cancer. Aberrant expression of WWP1 in gastric, prostate, and breast cancer, for instance, is an area of high interest, and therefore, WWP1 represents an excellent candidate for targeting in multiple cancer types.

**Application(s)**

- Screen molecules that inhibit WWP1 Ub ligase activity in HTS applications
- Determine Inhibitor $IC_{50}$
- Perform WWP1 real-time kinetic analyses.

**Supplied Materials**

| Catalog # | Name | Amount | Storage | |
|---|---|---|---|---|
| 80301 | UBE1 (UBA1), FLAG-tag (E1)* | 50 µg | -80°C | ***Avoid multiple freeze/ thaw cycles*** |
| 80314 | UbcH5b, His-Tag (E2)* | 60 µg | -80°C | |
| 80405 | WWP1, FLAG-tag (E3)* | 45 µg | -80°C | |
| 78307 | TRF Ubiquitin Mix (200x) | 50 µl | -80°C | |
| | ATP (4 mM) | 2 x 1 ml | -80°C | |
| | U2 Assay Buffer | 2 x 10 ml | -80°C | |
| | White, nonbinding Corning, 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. ***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 WWP1 intrachain TR-FRET Assay Kit is compatible with up to 1% final DMSO concentration. We recommend preparing the inhibitor in no higher than 5% DMSO solution in buffer and using 4 µl per well.

**Assay Protocol**

- All samples and controls should be performed in triplicate
- The assay should include a “Blank”, a “Positive control”, and a “Negative control”
- Calculate each protein, assay buffer, and ATP into single-use aliquots for the desired number of reactions per assay test.

1. Thaw **UBE1**, **UBCH5b**, **WWP1**, **TRF Ubiquitin Mix**, **U2 Assay Buffer**, and **ATP** on ice. Briefly spin the tubes to recover their full contents.

2. Prepare a 5-fold concentrated TRF Ubiquitin Mix in U2 Assay Buffer (prepare a 40-fold dilution of the provided 200x TRF Ubiquitin Mix).

3. Calculate the amount of protein required for the assay and prepare the appropriate amounts of diluted proteins. The concentration of each protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly.

    a. Dilute UBE1 in U2 Assay Buffer at 96 ng/µl (800 nM - the final concentration in the reaction is 40 nM)

    b. Dilute UBCH5b in U2 Assay Buffer 2 at 144 ng/µl (2 µM - the final concentration in the reaction is 100 nM)

    c. Dilute WWP1 in U2 Assay Buffer at 21.2 ng/µl (200 nM - the final concentration in the reaction is 50 nM);

    ***Keep all diluted proteins on ice until use. Do not freeze and re-use the diluted proteins.***

    *Note: Aliquot the remaining unused, undiluted protein into 2-4 aliquots as may be necessary (single use aliquots) and store them at -80°C. Aliquot assay buffer and ATP and store at -80°C.*

4. 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.

    *Without DMSO*

    a. If the Test Inhibitor is soluble in water, prepare a solution of the compound in U2 Assay Buffer that is 5-fold higher than the final desired concentration.

    ***Or***

    *With DMSO*

    a. If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 20-fold in U2 Assay Buffer (at this step the compound concentration is 5-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 5%.

    b. Prepare serial dilutions of the Test Inhibitor at concentrations 5-fold higher than the desired final concentrations using 5% DMSO in U2 Assay Buffer to keep the concentration of DMSO constant.

    c. 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).

5. To the wells designated as “Blank”, add 4 µl of **5x TRF Ubiquitin Mix** + 1 µl of **UBE1** + 1 µl of **UBCH5b** + 4 µl of **diluent solution** (for example DMSO 5%) + 5 µl of **U2 Assay Buffer.**

| Component | Blank |
|---|---|
| TRF Ubiquitin Mix (5x) | 4 µl |
| UBE1 | 1 µl |
| UBCH5b | 1 µl |
| WWP1 | - |
| Test Compound | - |
| Diluent solution* (no inhibitor) | 4 µl |
| U2 Assay Buffer | 5 µl |
| ATP (4 mM) | 5 µl |
| **Total** | **20 µl** |

**The diluent solution contains the assay buffer with the same concentration of solvent (e.g., DMSO) as the test compound solution.*

6. Prepare a Master Mix using diluted reagents: N wells × (4 µl of **5x TRF Ubiquitin Mix** + 1 µl of **UBE1** + 1 µl of **UBCH5b** + 5 µl of **WWP1**).

7. Add 11 µl of Master Mix to each well designated for the "Negative Control", "Positive Control", "Test Inhibitor".

8. Add 4 µl of Test Inhibitor to each well designated "Test Inhibitor". For "Positive Control" and "Negative Control", add 4 µl of the diluent solution without inhibitor.

9. Initiate the reaction by adding 5 µl of **ATP** (4 mM) to the wells labeled "Positive Control", "Test Inhibitor," and "Blank." Add 5 µl of **U2 Assay Buffer** to the well designated "Negative Control." Cover the plate with a plate sealer. Incubate the reaction at room temperature for two hours or at 30°C for one hour.

| Component | Test Inhibitor | Negative Control | Positive Control |
|---|---|---|---|
| Master Mix | 11 µl | 11 µl | 11 µl |
| Test compound | 4 µl | – | – |
| Diluent solution* (no inhibitor) | – | 4 µl | 4 µl |
| U2 Assay Buffer | – | 5 µl | – |
| ATP (4 mM) | 5 µl | – | 5 µl |
| **Total** | **20 µl** | **20 µl** | **20 µl** |

**The diluent solution contains the assay buffer with the same concentration of solvent (e.g., DMSO) as the test compound solution.*

Read the fluorescence intensity in a microtiter-plate reader capable of measuring TR-FRET.

***Note**: Two sequential measurements should be conducted. Eu-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). "Blank" value is subtracted from all other values.*

**Instrument Settings**

| Eu-donor emission | | Dye-acceptor emission | |
|---|---|---|---|
| Reading Mode | Time Resolved | Reading Mode | Time Resolved |
| Excitation Wavelength | 317±20 nm | Excitation Wavelength | 317±20 nm |
| Emission Wavelength | 620±10 nm | Emission Wavelength | 665±10 nm |
| Lag Time | 60 µs | Lag Time | 60 µs |
| Integration Time | 500 µs | Integration Time | 500 µs |

**Calculating Results**

Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission). "Blank" value is subtracted from all other values.

When percentage activity is calculated, the FRET value from the Blank (it is expected that Blank and Negative Control represent similar value) 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 FRETs = Sample FRET, $FRET_{blank}$ = Blank FRET, and $FRET_P$ = Positive control FRET.

**Example Results**

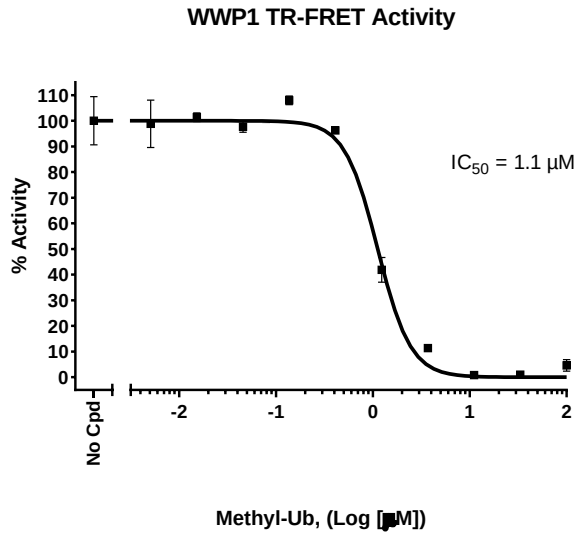


*Figure 1: WWP1 TR-FRET Activity.*
WWP1 TR-FRET activity was measured by inhibition of WWP1 auto-ubiquitination with Methylated Ubiquitin, using the WWP1 intrachain TR-FRET Assay Kit (BPS Bioscience #78554).

*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 all further questions, please email support@bpsbioscience.com

**Related Products**

| Products | Catalog # | Size |
|---|---|---|
| Cereblon intrachain TR-FRET Assay Kit | 78301 | 384 reactions |
| Cereblon Ubiquitination Homogenous Assay Kit | 79881 | 384 reactions |
| MDM2 intrachain TR-FRET Assay Kit | 78302 | 384 reactions |
| MDM2 TR-FRET Assay Kit | 79773 | 384 reactions |
| SMURF1 intrachain TR-FRET Assay Kit | 78303 | 384 reactions |
| VHL intrachain TR-FRET Assay Kit | 78305 | 384 reactions |
| XIAP intrachain TR-FRET Assay Kit | 78306 | 384 reactions |
| CBL-B TR-FRET Assay Kit | 79575 | 384 reactions |
| c-CBL TR-FRET Assay Kit | 79786 | 384 reactions |
| UBCH13 TR-FRET Assay Kit | 79741 | 384 reactions |
| UBCH5a TR-FRET Assay Kit | 79900 | 384 reactions |
| UBCH5c TR-FRET Assay Kit | 79901 | 384 reactions |
| UBCH5b TR-FRET Assay Kit | 79896 | 384 reactions |