**Description**

The UbcH6 (Ubiquitin-conjugating enzyme E2) TR-FRET Assay Kit is a homogeneous, sensitive TR-FRET assay designed to measure ubiquitination activity in a 384-reaction format. It utilizes biotin-labeled Ubiquitin and a Terbium-labeled antibody recognizing the His-tagged UbcH6 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. The kit contains enough purified UbcH6 (His-Tag), purified UBE1, Biotin-Ubiquitin, anti-His Tb-labeled donor, dye-labeled streptavidin acceptor, and assay buffer for 400 reactions.

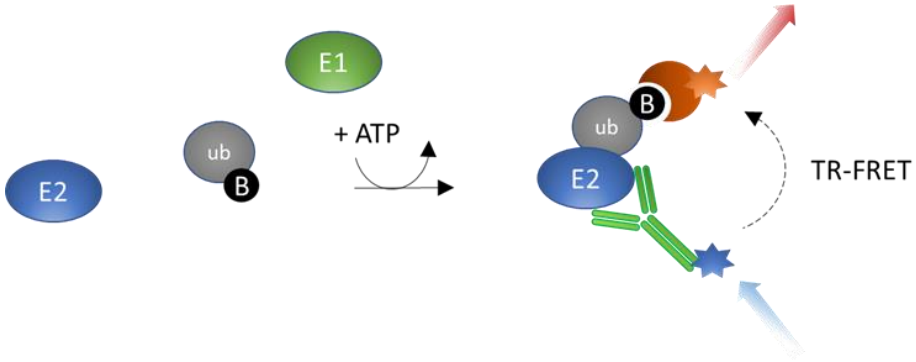


*Figure 1: UbcH6 TR-FRET Assay Kit schematic.*

The Terbium-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, and the TR-FRET signal can be measured using a fluorescence plate reader capable of measuring Time Resolved-Fluorescence Resonance Energy Transfer.

**Background**

UbcH6 (also known as Ubiquitin-conjugating enzyme E2 E1, UBE2E1) is an E2 ubiquitin-conjugating protein that receives Ubiquitin from a Ubiquitin-activating enzyme (E1) in an ATP-dependent fashion and transfers it to an E3 ligase. UbcH6 is regulated by Ubiquitin-specific protease 7 (USP7) and it is part of PRC1 (Polycomb Repressive Complex 1), an E3 complex that ubiquitinates histone H2 and it is thus involved in the proliferation of stem cells and cancer cells. It also interacts with and ubiquitinates ataxin-1, regulating its activity. Ataxin-1 is involved in spinocerebellar ataxia type 1 (SCA1), an autosomal-dominant neurodegenerative disease. Targeting UbcH6 may have therapeutical potential for the treatment of cancer and SCA1.

**Applications**

Screen molecules that inhibit UbcH6 activity in drug discovery and high throughput screening (HTS) applications.


BPS Bioscience
Our products are for research use only, not for diagnostic or therapeutic use
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 80301 | UBE1, FLAG-Tag* | 12 µg | -80°C |
| 80316 | UbcH6 (UBE2E1), His-Tag* | 5 µg | -80°C |
| | Biotin-Ubiquitin | 400 µl | -80°C |
| | ATP (10 mM) | 400 µl | -80°C |
| | 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 |
| | 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. ***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 UbcH6 TR-FRET Assay Kit is compatible with up to 1% final DMSO concentration. We recommend preparing the inhibitor in no higher than 10% DMSO solution in U2 Assay Buffer and using 2 µl per well.

**Assay Protocol**

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



Our products are for research use only, not for diagnostic or therapeutic use
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

1) Thaw **UBE1**, **UbcH6**, **U2 Assay Buffer**, **Biotin-Ubiquitin**, and **ATP** on ice. Briefly spin the tubes to recover their full content.

   *Note: UBE1, UbcH6, Biotin-Ubiquitin, ATP and U2 assay buffer are sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*

2) Dilute **UBE1** in U2 Assay Buffer to 20 ng/µl (1.5 µl/well).

3) Dilute **UbcH6** in U2 Assay Buffer to 8 ng/µl (1.5 µl/well).

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

4) Prepare an **Enzyme Mix**: N wells x (1.5 µl of UBE1 + 1.5 µl of UbcH6 + 1 µl of U2 Assay Buffer).

5) Add 4 µl of Enzyme Mix to the "Positive Control", "Negative Control" and "Test Inhibitor".

6) Add 4 µl of U2 Assay Buffer to the "Blank".

7) Prepare a **Substrate Mix** (4 µl/well)**:** N wells x (1 µl of ATP + 1 µl of Biotin-Ubiquitin + 2 µl of U2 Assay Buffer).

8) Add 4 µl of Substrate Mix to the "Blank", "Positive Control"" and "Test Inhibitor".

9) Prepare an **ATP-Deficient Substrate Mix** (4 µl/well): N wells x (1 µl of Biotin-Ubiquitin + 3 µl of U2 Assay Buffer).

10) Add 4 µl of ATP-Deficient Substrate Mix to "Negative control".

11) 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.

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

    b) If the Test inhibitor is soluble in DMSO, prepare it 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) so that all wells contain the same amount of DMSO. The final concentration of DMSO should not exceed 1%.

12) Add 2 µl of inhibitor solution to "Test Inhibitor".



Our products are for research use only, not for diagnostic or therapeutic use
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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

14) Dilute together the Anti-His Tb-labeled donor (1:400) and Dye-labeled acceptor (1:400) in U2 Assay Buffer (10 µl/well). This is the **Donor/Acceptor Mix**.

15) Add 10 µl of Donor/Acceptor Mixture to each well.

| | **Blank** | **Test Sample** | **Positive Control** | **Negative Control** |
|---|---|---|---|---|
| Enzyme Mix | - | 4 µl | 4 µl | 4 µl |
| U2 Assay Buffer | 4 µl | - | - | - |
| Substrate Mix | 4 µl | 4 µl | 4 µl | - |
| ATP-Deficient Substrate Mix | - | - | - | 4 µl |
| Test Inhibitor | - | 2 µl | - | - |
| Diluent Solution | 2 µl | – | 2 µl | 2 µl |
| Donor/Acceptor Mix | 10 µl | 10 µl | 10 µl | 10 µl |
| **Total** | **20 µl** | **20 µl** | **20 µl** | **20 µl** |

16) Protect from light and incubate the reaction at Room Temperature for 40 minutes or perform kinetic analysis.

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

18) The "Blank" value should be 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 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).

To calculate the percentage activity substrate the Blank value from all other FRET values. It is expected that Blank and Negative Control have a similar value. The FRET value from the Positive Control can be set as one hundred percent activity as it corresponds to the maximum activity.



BPS Bioscience

Our products are for research use only, not for diagnostic or therapeutic use

● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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

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

**Example Results**

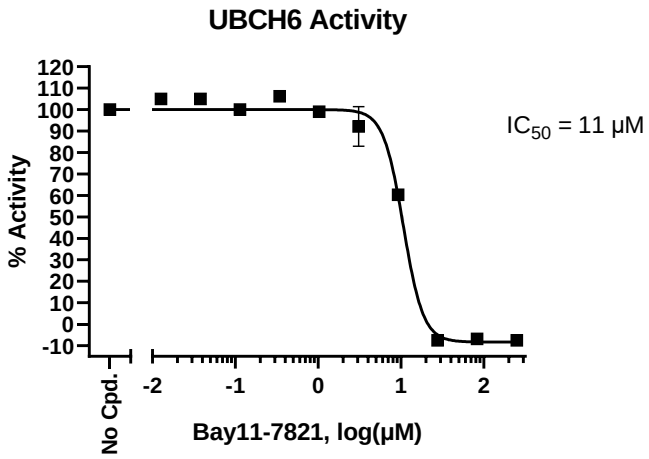


*Figure 2: UbcH6 activity is inhibited by Bay11-7821.*
UbcH6 activity was measured in the presence of increasing concentrations of Bay11-7821 inhibitor (Tocris #1744). Results are expressed as percentage of activity relative to positive control (measured in the absence of inhibitor and set at 100%).

*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 further questions, email support@bpsbioscience.com

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| UbcH6 (UBE2E1), His-Tag Recombinant | 80316 | 100 µg |
| UBE1 (UBA1), FLAG-Tag Recombinant | 80303 | 100 µg |
| ChoosE3-Freedom™ Intrachain TR-FRET Assay Kit | 78560 | 384 reactions |
| ChoosE2-Opti™ Intrachain TR-FRET Assay Kit | 78561 | 384 reactions |


Our products are for research use only, not for diagnostic or therapeutic use
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com