Description

The UBE1 Inhibitor Screening Assay Kit is a 96-well format assay designed to measure the activity of the ubiquitin activating enzyme UBE1 for screening and profiling applications, using the Kinase-Glo® MAX as detection reagent. The kit contains enough purified UBE1 protein, Ubiquitin, ATP, and kinase assay buffer for 100 reactions.

Background

UBE1 (also known as Ubiquitin Activating enzyme 1, UBA1 or Ubiquitin like modifier Activating enzyme 1) belongs to the family of ubiquitin activating enzymes, responsible for the first reaction in the multi-step protein ubiquitination process. UBE1 forms a thioester bond with ubiquitin in an ATP dependent reaction. In humans only two ubiquitin activating enzymes are known, UBE1 and UBA6 and through their role in the ubiquitination pathway, they are involved in the regulation of several critical cellular processes, such as cell cycle and signal transduction. Mutations in UBE1 have been identified in spinal muscular atrophy and cancer. UBE1 inhibitors can prove crucial for the treatment of neurodegenerative diseases and cancer.

Applications

Enzyme kinetics and screening small molecule inhibitors for drug discovery and high-throughput screen (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
80301	UBE1, FLAG-Tag*	150 µg	-80°C
79334	5x Kinase Assay Buffer	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
	Ubiquitin (500 µM)	100 µl	-20°C
79696	96-well white microplate	1	Room Temp

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

Materials Required but Not Supplied

Kinase-Glo® MAX (Promega #V6071)
Dithiothreitol (DTT, 0.5 M; optional)
Adjustable micropipettor and sterile tips
Luminescence Plate Reader
30°C incubator

Stability

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.

Assay Protocol

- All samples and controls should be performed in duplicates.
- The assay should include a "Negative Control", a "Positive Control," and a "Test inhibitor."
- If the assay plate is going to be used more than once, prepare enough of each reagent 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 as recommended for each reagent. ***Unused diluted proteins should be discarded.***

1. Thaw **5x Kinase Assay Buffer, ATP (500 µM) and Ubiquitin (500 µM).**

   Optional: If desired, add 30 µl of DTT 0.5 M to 1.5 ml of 5x Kinase Assay Buffer.

2. Prepare a Master Mix (25 µl/well): N well x (10 µl 5x Kinase Assay Buffer + 0.5 µl ATP (500 µM) + 1 µl Ubiquitin + 13.5 µl distilled water).

3. Add 25 µl of Master Mix to each well.

4. Prepare 3 ml of 1x Kinase Assay Buffer by mixing 600 µl of 5x Kinase Assay Buffer with 2400 µl of distilled water. 3 ml of 1x Kinase Assay Buffer is sufficient for 100 reactions.

5. Prepare the Test inhibitor (5 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 50 µl.

   4.1. If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x Assay Buffer at concentrations 10-fold higher than the desired final concentrations. The 1x Kinase Assay Buffer is the Diluent Solution.

   OR
   7.2. 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 final concentration, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using 1x Kinase Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in 1x Kinase Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

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

6. Add 5 µl of Test inhibitor to each well designated "Test Inhibitor".

7. Add 5 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

8. Thaw **UBE1, FLAG-Tag** on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

9. Dilute UBE1 to 75 ng/µl with 1x Kinase Assay Buffer. You will need 20 µl per well. Aliquot any unused (non-diluted) UBE1 into single use aliquots and store at -80°C.

   Note: The concentration of UBE1 provided may vary. Verify the concentration of the UBE1 written on the tube and dilute accordingly. Prepare only the amount required for the assay.

   UBE1 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots and do not re-use the diluted protein.

10. Add 20 µl of 1x Kinase Assay Buffer to the "Negative Control" wells.

11. Initiate the reaction by adding 20 µl of diluted UBE1 to the "Positive Control" and "Test Inhibitor" wells.

    Protect your samples from direct exposure to light and incubate at Room Temperature for 30 minutes.

Component	Negative control	Positive Control	Test Inhibitor
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted UBE1 (75 ng/µl)	-	20 µl	20 µl
1x Kinase Assay Buffer	20 µl	-	-
Total	**50 µl**	**50 µl**	**50 µl**

12. Incubate for 30°C for 45 minutes.

13. Thaw Kinase-Glo® MAX reagent.

14. Add 50 µl of Kinase-Glo® MAX reagent to each well.

15. Cover with foil and incubate at Room Temperature for 15 minutes.

16. Read the luminescence value of the samples.

17. Subtract the Negative Control value from all other conditions.

Example Results

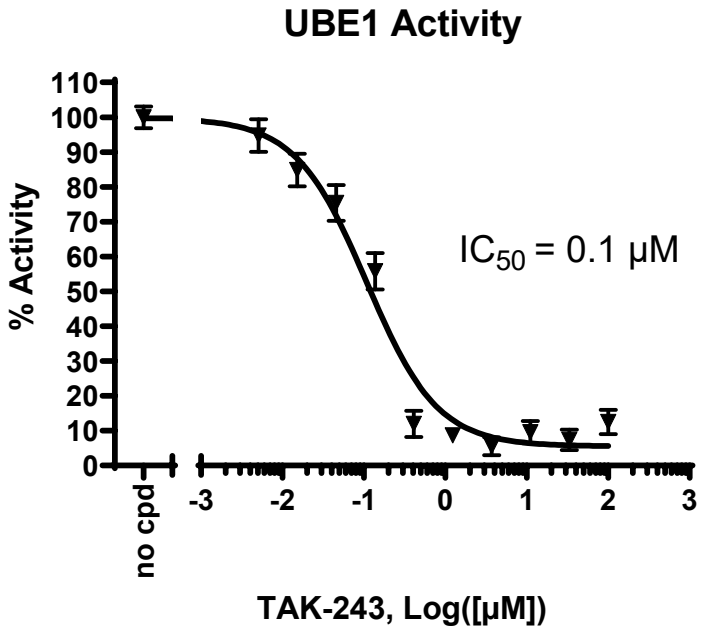

*Figure 1. UBE1 activity is inhibited by TAK243.*UBE1 activity was measured in the presence of increasing concentrations of TAK243. Results are expressed as percentage of activity relative to positive control (measured in the absence of inhibitor and set at 100%).

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, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
UBE1 (UBA1), FLAG-tag Recombinant	80301	100 µg/1 mg
Ubiquitin, His-tag	79293	2 mg
UBA6 (UBE1L2), FLAG-tag Recombinant	80303	100 µg