Description

The ULK1 Kinase Assay Kit is designed to measure ULK1 activity for screening and profiling applications, using Kinase-Glo® MAX as a detection reagent. The ULK1 Assay Kit comes in a convenient 96-well format, with enough purified ULK1, MBP substrate, ATP, and kinase assay buffer for 96 enzyme reactions.

Background

ULK1 (unc-51 like autophagy activating kinase 1) is a serine/threonine protein kinase that plays a critical role during the initial stages of autophagy in response to nutrient starvation. The conserved C-terminal domain of ULK1 controls the function and the localization of the protein. Knockdown of ULK1 inhibits the autophagic response and prevents rapamycin-induced autophagy, consistent with a role downstream of mTOR. ULK1 forms a complex with FIP200 and ATG13 and this complex is essential for starvation-induced autophagy (1). Both FIP200 and ATG13 are critical for correct localization of ULK1 to the pre-autophagosome and stability of the ULK1 protein (2). ULK1 is phosphorylated upon activation of the mTOR pathway in a nutrient starvation-regulated manner. Alterations in ULK signaling pathways may be involved in the formation of autophagy-regulated Lewi bodies, which have been associated with Parkinson's disease (3).

Applications

Study enzyme kinetics and screen small molecular inhibitors for drug discovery and High Throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
40099	ULK1*	20 µg	-80°C
79334	Kinase Buffer 1 (5X)	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
40535	Myelin basic protein (MBP), 5 mg/ml	200 µl	-20°C
79696	96-well plate, white	1	Room Temp.

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

Materials Required but Not Supplied

Name	Catalog #
Kinase-Glo MAX	Promega, #V6071
Microplate reader capable of reading luminescence	-
Adjustable micropipettor and sterile tips	-
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 Principle

Kinase activity is measured using Kinase-Glo™ Max (Promega; #V6071). The addition of the reagent results in the generation of a luminescent signal that correlates with the amount of ATP. The reagent is linear to 100µM ATP.

Contraindications

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

Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw 5x Kinase assay buffer, ATP (500 µM), and MBP (5 mg/ml).
2. Prepare the Master Mix (25 µl/well): N wells x (5 µl of 5x Kinase assay buffer + 1 µl of ATP (500 µM) + 2 µl of MBP (5 mg/ml) + 17 µl of distilled water). Add 25 µl to every well.
3. Prepare 3 ml of 1x Kinase assay buffer by mixing 600 µl of 5x Kinase assay buffer with 2400 µl distilled water. Three (3) ml of 1x Kinase assay buffer is sufficient for 100 reactions.
4. 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.

   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x Kinase assay buffer , 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x Kinase assay buffer (Diluent Solution).

   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x Kinase assay buffer to prepare the highest concentration of the 10-fold intermediate solution. 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 1x Kinase assay buffer to keep the concentration of DMSO constant.

   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).

5. Add 5 µl of 10x Test Inhibitor solution to each well labeled as “Test Inhibitor.”

   For the “Positive Control" and “Blank,” add 5 µl of Diluent Solution (either 1x Kinase assay buffer or 10% DMSO in 1x Kinase assay buffer, as described above).

6. Thaw ULK1 on ice. Briefly spin the tube containing the enzyme to recover its full contents. Dilute the enzyme to 10 ng/µl using 1x Kinase assay buffer.

   Note: ULK1 is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots and do not re-use the diluted enzyme.

7. Initiate the reaction by adding 20 µl of diluted ULK1 to the wells designated “Positive Control” and “Test Inhibitor Control.”

   To the wells designated as "Blank," add 20 µl of 1x Kinase assay buffer.

Component	Positive Control	Test Inhibitor	Blank
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	5 µl	-
Diluent buffer (no inhibitor)	5 µl	-	5 µl
1x Kinase assay buffer	-	-	20 µl
ULK1 (10 ng/µl)	20 µl	20 µl	-
Total	50 µl	50 µl	50 µl

8. Incubate at 30°C for 45 minutes.
9. During the incubation, thaw the Kinase-Glo Max reagent. After the 45-minute reaction, add 50 µl of Kinase-Glo Max reagent to each well. Cover the plate with aluminum foil and incubate the plate at room temperature for 15 minutes.
10. Immediately read in a luminometer or a microplate reader capable of reading luminescence. The “Blank” value is subtracted from all other readings.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example of Results:

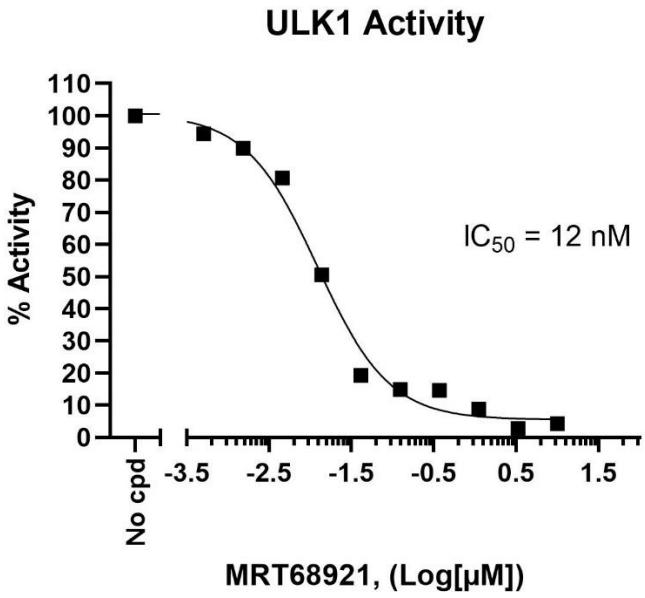

Figure 1: Inhibition of ULK1 kinase activity by MRT68921. ULK1 activity was measured in the presence of increasing concentrations of MRT68921 using the ULK1 Kinase Assay Kit (BPS Bioscience #78362).

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.

References

1. Ganley I.G. *et al., J. Biol. Chem.* **284 (18):** 12297-12305 (2009).
2. Chan E.Y. *et al*., *Mol. Cell. Biol.* **29 (1):** 157-171 (2009).
3. Miki Y. *et al.* Alteration of Upstream Autophagy-Related Proteins (ULK1, ULK2, Beclin1, VPS34 and AMBRA1) in Lewy Body Disease. *Brain Pathol.* 2016; **26**: 359-70.

Related Products

Products	*Catalog #*	*Size*
ULK1, FLAG-tag	40099	10 µg
ULK2, GST-tag	40294	10 µg
ULK3, His-tag	40295	10 µg
Kinase Buffer 1	79344	10 ml
ATP (500 µM)	79686	100 µl