ULK1, Active
Recombinant human protein expressed in Sf9 cells

Catalog # U01-11G
Lot # G1163-1

Product Description
Recombinant human ULK1 (1-649) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is BC111603.

GeneAliases
ATG1, UNC51, Unc51.1, FLJ38455

Formulation
Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability
Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Scientific Background
ULK1 is a serine/threonine protein kinase that plays critical role during initial stages of autophagy which is a vital response to nutrient starvation. The conserved C-terminal domain (CTD) of ULK1 controls the regulatory function and localization of the protein. Knockdown of ULK1 inhibits the autophagic response as well as inhibiting rapamycin-induced autophagy consistent with a role downstream of mTOR (1). ULK1 forms a complex with FIP200 and ATG13 and this complex is essential for starvation-induced autophagy (2). Both FIP200 and ATG13 are critical for correct localization of ULK1 to the pre-autophagosome and stability of ULK1 protein. ULK1 is phosphorylated by the mTOR pathway in a nutrient starvation-regulated manner.

References

Specific Activity
The specific activity of ULK1 was determined to be 56 nmol/min/mg as per activity assay protocol.

Purity
The purity of ULK1 was determined to be >70% by densitometry, approx. MW ~125kDa.

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Catalog # Specific Activity
U01-11G 56 nmol/min/mg

Lot # Stability
G1163-1 Storage & Shipping

Concentration
0.1 µg/µl

Stability
1yr at –70°C from date of shipment

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.
Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: U01-11G)
Active ULK1 (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active ULK1 for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)
Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)
Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

Substrate (Catalog #: M42-51N)
Myelin Basic Protein (MBP) substrate diluted in distilled H₂O to a final concentration of 1mg/ml.

[³³P]-ATP Assay Cocktail
Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)
Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at –20°C.

Assay Protocol

Step 1. Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
Step 2. Thaw the Active ULK1, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
  Component 1. 10µl of diluted Active ULK1 (Catalog #U01-11G)
  Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
  Component 3. 5µl distilled H₂O (4°C)
Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
Step 5. Initiate the reaction by the addition of 5 µl [³³P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P³³]-ATP Specific Activity (SA) (cpm/pmol)
Specific activity (SA) = cpm for 5 µl [³³P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)
Corrected cpm from reaction / [(SA of [³³P]-ATP in cpm/pmol) *(Reaction time in min) *(Enzyme amount in µg or mg)]*[ (Reaction Volume) / (Spot Volume)]

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