SignalChem
Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

ASK1, Active

Recombinant human protein expressed in Sf9 cells

Catalog # M13-11G-10

Lot # U315-1

Product Description

Recombinant human ASK1 (649-946) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_005923.

Gene Aliases

MAP3K5; MEKK5; MAPKKK5

Concentration

0.1 µg/µl

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, Shipping 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.
Stability is 1yr at –70°C from date of shipment. Product shipped on dry ice.

Scientific Background

ASK1, also known as MAPKKK5, activates MKK3, MKK4 (SEK1), and MKK6. Overexpression of ASK1 induces apoptotic cell death, and ASK1 is activated in cells treated with tumor necrosis factor-alpha (1). ASK1 interacts with members of the TRAF family and is activated by TRAF2 in the TNF-signaling pathway. After activation by TRAF2, ASK1 activates MKK4, which in turn activates JNK. Thus, ASK1 is a mediator of TRAF2-induced JNK activation (2).

References

1. Ichijo, H. et al: Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 275: 90-94, 1997.
2. Nishitoh, H. et al: ASK1 is essential for JNK/SAPK activation by TRAF2. *Molec. Cell* 2: 389-395, 1998.

Purity

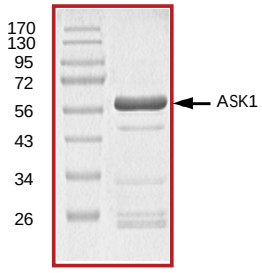
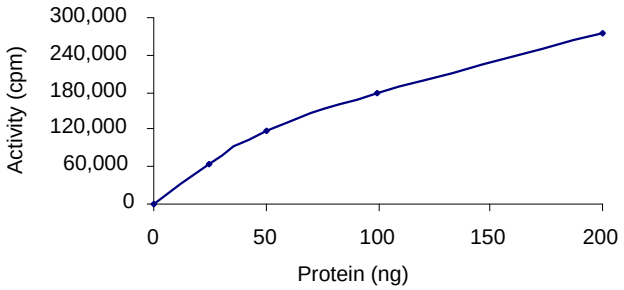


Figure 1. SDS-PAGE gel image

The purity was determined to be **>90%** by densitometry.
Approx. MW **60kDa**.

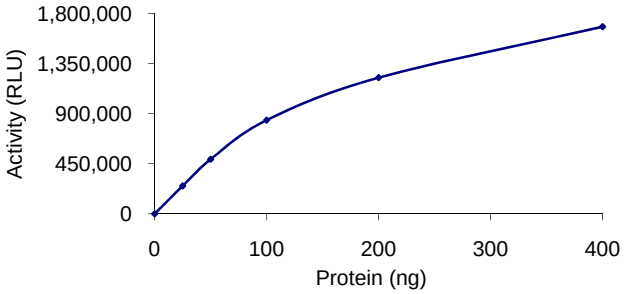
Specific Activity

Figure 2. Radiometric Assay Data



The specific activity of ASK1 was determined to be **96 nmol /min/mg** as per activity assay protocol.
(For Radiometric Assay Protocol on this product please see pg. 2)

Figure 3. ADP- Glo™ Assay Data



The specific activity of ASK1 was determined to be **130 nmol /min/mg** as per activity assay protocol.
(For ADP-Glo™ Assay Protocol on this product please see pg. 3)

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M13-11G-10)

Active ASK1 (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 ASK1 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 50 ng/μl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{32}P]-ATP Assay Cocktail

Prepare 250μM [^{32}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{32}P]-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.

Substrate (Catalog #: M42-51N)

Myelin Basic Protein (MBP) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{32}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active ASK1, 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 ASK1 (Catalog #M13-11G-10)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
- **Component 3.** 5μl distilled H_2O (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 H_2O.

Step 5. Initiate the reaction by the addition of 5μl [^{32}P]-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 20μl 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 H_2O) 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 (cpm) 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 [P^{32}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5μl [^{32}P]-ATP / pmoles of ATP (in 5μl of a 250μM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{32}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

ADP-Glo™ Activity Assay Protocol

Reaction Components

ASK1 Kinase Enzyme System (Promega, Catalog #:V3881)

ASK1, Active, 10µg (0.1µg/µl)
MBP Protein, 1ml (1mg/ml)
Reaction Buffer A (5X), 1.5ml
DTT solution (0.1M), 25µl

ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP, 10 mM (0.5ml)
ADP, 10 mM (0.5ml)
ADP-Glo™ Reagent (5ml)
Kinase Detection Buffer (10ml)
Kinase Detection Substrate (Lyophilized)

Reaction Buffer A (5X)

200mM Tris-HCl, pH 7. 5, 100mM $MgCl_2$ and 0.5 µg/µl BSA.

Assay Protocol

The ASK1 assay is performed using the ASK1 Kinase Enzyme System (Promega; Catalog #: V3881) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The ASK1 reaction utilizes ATP and generates ADP. Then the ADP- Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding t*he ADP-Glo™ Kinase Assay,* see the technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html.

Step 1. Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.

Step 2. Thaw the components of ASK1 Enzyme System, ADP and ATP on ice.

Step 3. Prepare 1ml of 2X Buffer by combining 400µl Reaction Buffer A, 1µl DTT and 599µl of dH_20.

Step 4. Prepare 1ml of 250µM ATP Assay Solution by adding 25µl ATP solution (10mM) to 500µl of 2X Buffer and 475µl of dH_20.

Step 5. Prepare diluted ASK1 in 1X Buffer (diluted from 2X buffer) 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 ASK1 for optimal results).

Step 6. In a white 96-well plate (Corning Cat # 3912), add the following reaction components bringing the initial reaction volume up to 20µl:

Component 1.	10µl of diluted Active ASK1
Component 2.	5µl of 1mg/ml stock solution of substrate
Component 3.	5µl of 2X Buffer

Step 7. Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 8. At the same time as the ASK1 kinase reaction, set up an ATP to ADP conversion curve at 50µM ATP/ADP range as described in the *ADP-Glo™ Kinase Assay* technical Manual #TM313.

Step 9. Initiate the ASK1 reactions by the addition of 5µl of 250 µM ATP Assay Solution thereby bringing the final volume up to 25µl. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.

Step 10. Terminate the reaction and deplete the remaining ATP by adding 25µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.

Step 11. Add 50µl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.

Step 12. Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® Microplate Luminometer (Promega; Cat # E6501).

Step 13. Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLUs, see ADP-Glo™ Applications Database at http://www.promega.com/applications/cellularanalysis/cellsignaling.htm

Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol) / (Reaction time in min)*(Enzyme amount in mg)