

Product Specification

ASK1, active

(Recombinant human protein, residues 649-946, expressed in Sf 9 cells)

Catalog #: 7710-5
 Lot #: _____
 Aliquot size: 5 µg protein in 50 µl
 Specific activity: 97 nmol/min/mg

Quality Control Analysis

Activity assessment

ASK1 protein (100 ng/µl concentration) was diluted to 50ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT and 40ng/µl BSA), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the myelin basic protein (MBP) in the following assay condition:

- 10 µl diluted ASK1 protein
- 10 µl MBP (1 mg/ml stock)
- 5 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30°C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

Error!

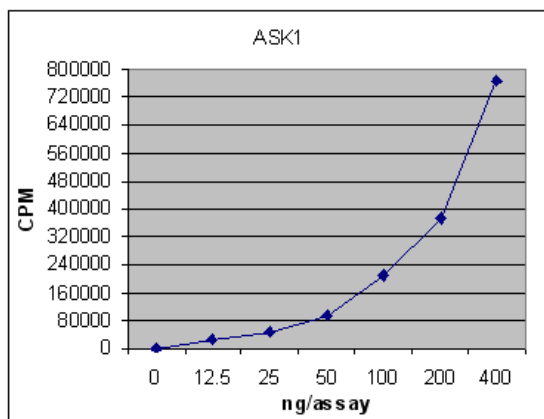


Fig. 1 ASK1 activity assay

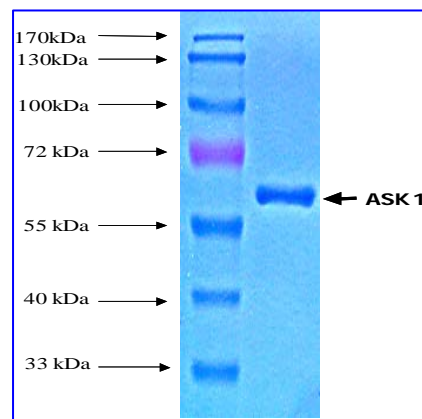


Fig. 2 ASK1 protein gel

Purity assessment

1 µg of ASK1 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >95% purity of the ASK1 product, and the band was at ~60 kDa (Fig. 2).

Product Description

Recombinant human ASK1 protein (649-946) containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is NM_005923.

This material is sold for research purposes only.

Specific Activity

97 nmol phosphate incorporated into MBP per minute per mg protein at 30°C for 15 minutes using a final concentration of 50 μM ATP (0.83 μCi/assay).

Formulation

Recombinant proteins in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

Storage and Stability

Store product frozen at or below -70°C. Stable for 1 year at -70°C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background

Mitogen-activated protein kinase (MAPK) signaling cascades include MAPK or ERK, MAPK kinase (MKK or MEK), and MAPK kinase kinase (MAPKKK or MEKK). MAPKK kinase/MEKK phosphorylates and activates its downstream protein kinase, MAPK kinase/MEK, which in turn activates MAPK. ASK1 (MAPKKK5) contains 1,374 amino acids with all 11 kinase subdomains. Northern blot analysis shows that MAPKKK5 transcript is abundantly expressed in human heart and pancreas. The MAPKKK5 protein phosphorylates and activates MKK4 (aliases SERK1, MAPKK4) in vitro, and activates c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK) during transient expression in COS and 293 cells; MAPKKK5 does not activate MAPK/ERK (1). ASK1 also 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 (2). 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 (3). Fas triggers cell death specifically in motor neurons by transcriptional upregulation of neuronal nitric oxide synthase (nNOS) mediated by p38 kinase. ASK1 and Daxx act upstream of p38 in the Fas signaling pathway, which was unique to motor neurons and may contribute to motor neuron loss in ALS. (4)

References

1. Wang, X. S.; Diener, K.; Jannuzzi, D.; Trollinger, D.; Tan, T.-H.; Lichenstein, H.; Zukowski, M.; Yao, Z.: Molecular cloning and characterization of a novel protein kinase with a catalytic domain homologous to mitogen-activated protein kinase kinase kinase. *J. Biol. Chem.* 271: 31607-31611, 1996.
2. Ichijo, H.; Nishida, E.; Irie, K.; ten Dijke, P.; Saitoh, M.; Moriguchi, T.; Takagi, M.; Matsumoto, K.; Miyazono, K.; Gotoh, Y.: Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 275: 90-94, 1997.
3. Nishitoh, H.; Saitoh, M.; Mochida, Y.; Takeda, K.; Nakano, H.; Rothe, M.; Miyazono, K.; Ichijo, H.: ASK1 is essential for JNK/SAPK activation by TRAF2. *Molec. Cell* 2: 389-395, 1998.
4. Raoul, C.; Estevez, A. G.; Nishimune, H.; Cleveland, D. W.; deLapeyriere, O.; Henderson, C. E.; Hasse, G.; Pettmann, B.: Motoneuron death triggered by a specific pathway downstream of Fas: potentiation by ALS-linked SOD1 mutations. *Neuron* 35: 1067-1083, 2002.