

## ASK1 (Human), Active

### Recombinant protein expressed in Sf9 cells

Cat# CY-SPM13

Lot No. B354-1  
5 µg 0.1 µg/µl

#### 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).

#### 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:

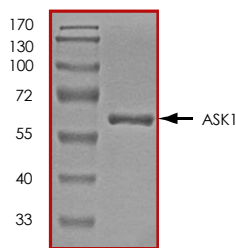
ASK1; MEKK5; MAPKKK5

#### Formulation:

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

#### Purity & Molecular Weight:

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



#### Storage:

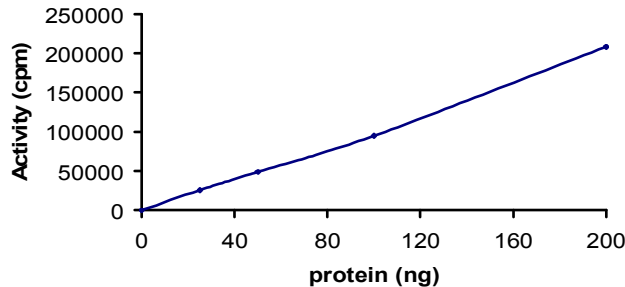
Store product at -70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

#### Stability:

Unopened vial at -70 °C, 1 year from date of shipment.

**Specific Activity:**

The specific activity was determined to be 97 nmol/min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of the kinase in a 25  $\mu$ L reaction consisting of 5  $\mu$ L of 5 X Kinase Assay Buffer, 10  $\mu$ L of 1 mg/ml the Substrate Solution, 5  $\mu$ L of diluted kinase and 5  $\mu$ L of 250  $\mu$ M ATP solution containing [ $\gamma$ - $^{32}$ P] ATP (0.167  $\mu$ Ci/ $\mu$ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20  $\mu$ L of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

**Substrate Solution:**

Myelin basic protein (MBP) diluted in distilled H<sub>2</sub>O to a final concentration of 1 mg/ml.

**5 X Kinase Assay Buffer:**

25mM MOPS, 12.5mM  $\beta$ -glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**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.

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