

# Recombinant Human Active ASK1 (649-946)

Certificate of Analysis

Catalog Number: 3575-KS

Lot Number: 1475430

## Specifications and Use

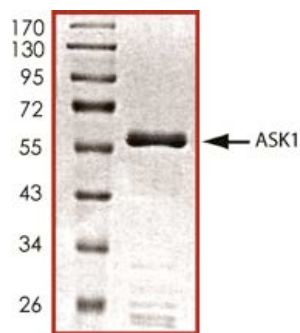
- Source** ♦ Recombinant human ASK1 (amino acids 649-946) was expressed by baculovirus in *Sf9* insect cells using an N-terminal GST tag. The gene accession number is NM\_005923.
- Molecular Mass** ♦ The approximate molecular weight is 60 kDa (see Figure 1 below).
- Purity** ♦ The purity was determined to be > 95% by densitometry (see Figure 1 below).
- Formulation** ♦ Supplied in 50 mM Tris HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 10 mM glutathione, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol.
- Size** ♦ 10 µg.
- Concentration** ♦ 0.1 µg/µL.
- Activity** ♦ The specific activity of ASK1 was determined to be 96 nmol/min/mg using a myelin basic protein (MBP) substrate (see activity assay protocol).
- Storage** ♦ This product is stable at ≤ -70 °C for up to 1 year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature.  
♦ **Avoid repeated freeze-thaw cycles.**

## ASK1

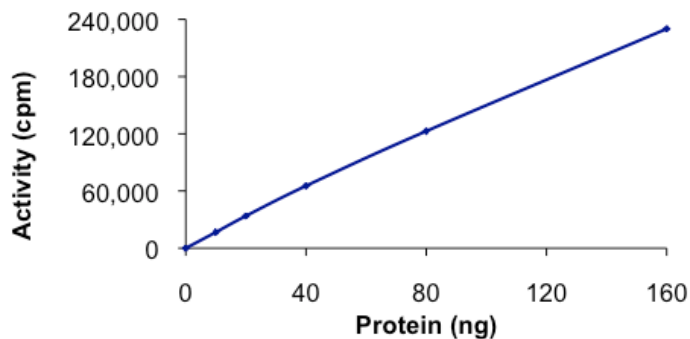
ASK1, also known as MEKK5 or MAPKKK5, activates MKK3, MKK4 (SEK1), and MKK6. Over-expression of ASK1 induces apoptotic cell death and is activated in cells treated with tumor necrosis factor alpha (TNF- $\alpha$ ) (1). ASK1 interacts with members of the TRAF family and is activated by TRAF2 in the TNF-signaling 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.* (1997) *Science* **275**:90.
2. Nishitoh, H. *et al.* (1998) *Molec. Cell* **2**:389.



**Figure 1:** SDS-PAGE and coomassie stain. The approximate molecular weight is 60 kDa and the purity is > 95%.



**Figure 2:** Enzymatic assay results. The specific activity of ASK1 was determined to be 96 nmol/min/mg as per activity assay protocol (on reverse).

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## Activity Assay Protocol

### Solutions Required

- **Active Kinase** - Active ASK1 (0.1 µg/µL) diluted with Kinase Dilution Buffer and assayed as outlined in Figure 2.  
**Note:** These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.
- **Kinase Assay Buffer I, pH 7.2** - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 25 mM MgCl<sub>2</sub>, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer, pH 7.2** - Kinase Assay Buffer I diluted at a 1:4 ratio (5-fold dilution) with 50 ng/µL BSA solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I.
- **[<sup>33</sup>P]-ATP Assay Cocktail** - Prepare 250 µM [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 µL of 10 mM ATP Stock Solution, 100 µL of [<sup>33</sup>P]-ATP (1 mCi/100 µL), and 5.75 mL of Kinase Assay Buffer I.
- **Substrate** - Myelin basic protein (MBP) diluted in distilled or deionized water to a final concentration of 1 mg/mL.

### Assay Procedure

1. Thaw the [<sup>33</sup>P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area.
2. Thaw the Active ASK1, Kinase Assay Buffer I, Substrate, and Kinase Dilution Buffer on ice.
3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µL.

Reaction Component	Amount
Diluted Active ASK1	10 µL
Substrate (1 mg/mL Stock Solution)	5 µL
Distilled H <sub>2</sub> O (2-8 °C)	5 µL

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 or deionized water.
5. Initiate the reaction by the addition of 5 µL [<sup>33</sup>P]-ATP Assay Cocktail, bringing the final volume up to 25 µL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
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.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10 mL of phosphoric acid and make a 1 liter solution with distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
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.

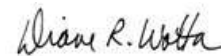
### Calculation of [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5 µL [<sup>33</sup>P]-ATP/pmole of ATP (in 5 µL of a 250 µM ATP stock solution, *i.e.* 1250 pmoles)

### Calculation of Kinase Specific Activity (SA) (pmol/minutes/µg or nmol/minutes/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in µg or mg)] x [(Reaction volume) / (Spot Volume)]

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Quality & Regulatory Affairs