

Product Specification Sheet

Product: IgG Fraction of Anti-ASK-1 Human Phospho S83 Specific [Rabbit]

Code: 200-401-410

Lot # 11375

Size: 200 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 2.0 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Application Note(s): This phospho specific polyclonal antibody reacts human pS83 ASK1 and shows minimal reactivity by western blot, ELISA and competitive ELISA with non-phosphorylated ASK1. Although not tested, this antibody is likely functional in RIA, immunohistochemistry and immunoprecipitation.

Background Information: ASK-1 (apoptosis signal-regulating kinase 1 - also referred to as MEK Kinase-5 or MAPKKK5) is a novel serine/threonine MAP kinase kinase kinase (MAPKKK) component of the mitogen -activated protein (MAP) cascade that is activated in response to extracellular stimuli by cytokines, growth factors and environmental stresses and other factors. Overexpression of ASK-1 induces apoptotic cell death. ASK-1 is expressed in a variety of human and mouse tissues. The overall amino acid sequence identity between the mouse and human ASK1 is 91.9%. ASK-1 interacts with CDKN1A (also known as p21, WAF1, CIP1). Please refer to the reference list at the end of this document for further information.

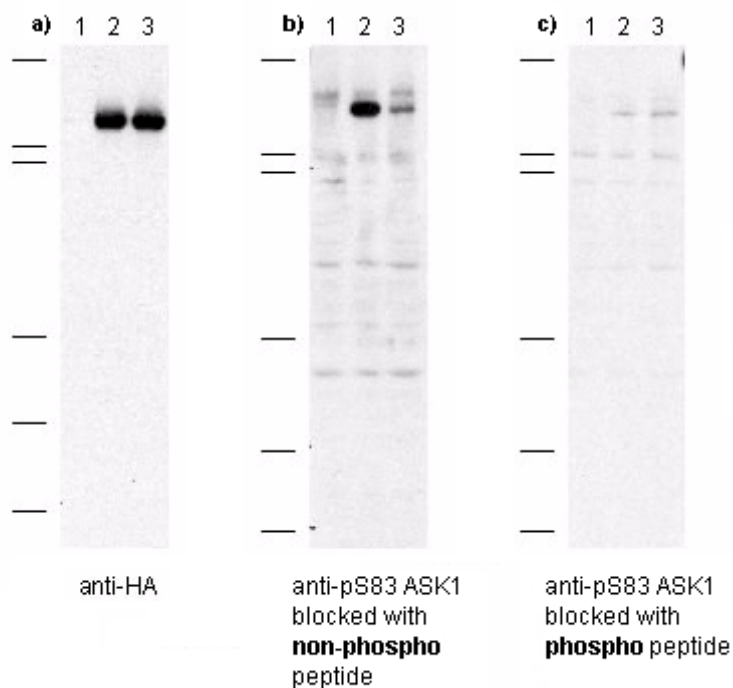
Figure 1. Immunoblot of anti-pS83 ASK1 antibodies shows specificity for phosphorylated human ASK1.

Anti-pS83 (aa 76-87) antibody, generated by immunization with phospho peptide coupled to KLH, was tested by immunoblot against lysates of Cos-7 cells after transient transfection, separately, with 1) vector only, 2) recombinant HA-ASK1, and 3) recombinant human HA-ASK1 where S83 was substituted with an alanine residue. Cells were lysed 24 h post-transfection in 200 µL of 1x SDS-sample buffer, heated at 96°C for 5', and vortexed for 30 sec. Samples (10 µL each) were separated on a 12% SDS-PAGE gel and transferred to PVDF (Millipore) followed by blocking for 45' using TTBS supplemented with 5% non-fat dry milk. All incubations were performed at room temperature.

In panel a) all samples were incubated with 10 µg/mL mouse anti-HA antibody for 45'. After 5X washes with TTBS, reaction with ALP rabbit anti-mouse IgG at 200 ng/mL proceeded for 45' following again by washing as before. The blot was developed using BCIP/NBT. This blot demonstrates both recombinant transfections were successfully over-expressed in the Cos-7 cells.

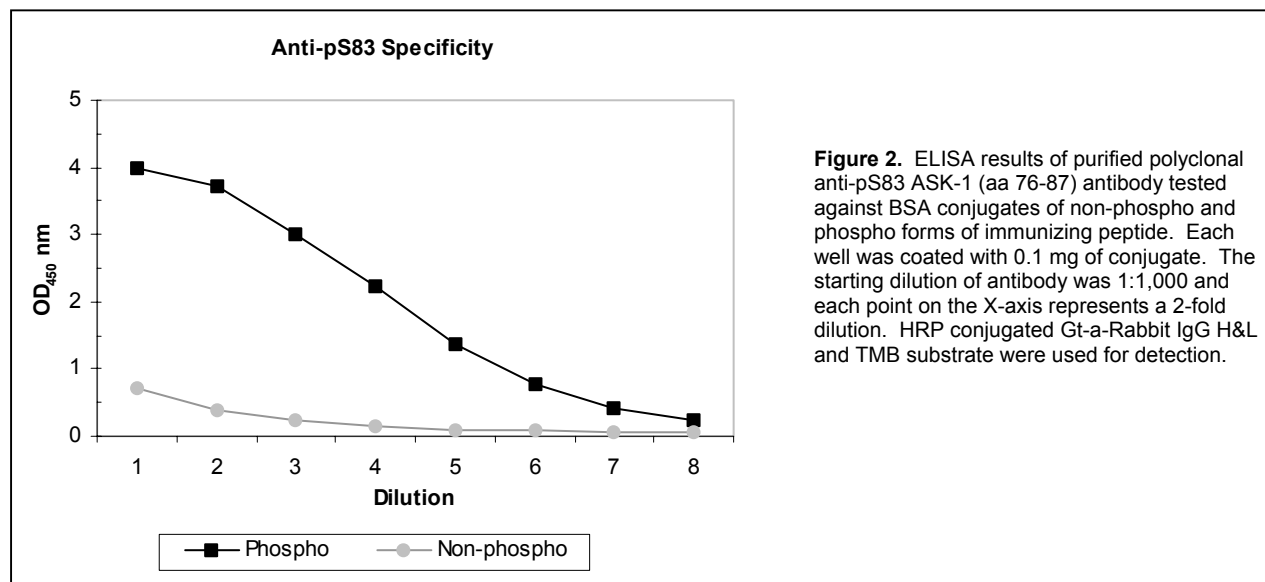
In panel b) all samples were incubated with a 1:1,000 dilution of Rockland's anti-pS83 ASK1 antibody for 45'. The antibody was pre-incubated with non-phospho peptide prior to membrane incubation. After 5X washes with TTBS, reaction with HRP goat anti-rabbit IgG at 10 ng/mL proceeded for 45' following again by washing as before. The membrane was processed as before. Lane 2 shows strong specific staining of ASK1. Lane 3, where S83 was replaced with alanine, shows greatly diminished staining.

In panel c) all samples were incubated with a 1:1,000 dilution of Rockland's anti-pS83 ASK1 antibody as before except the antibody was pre-incubated with phospho peptide prior to membrane incubation. No staining is observed after phospho peptide blocking occurs.



Recommended Dilution(s): For immunoblotting a 1:1,000 dilution is recommended. A 155 kDa band corresponding to human ASK-1 is detected. Whole cell lysates from SW1353 can be used as a positive control. For ELISA a 1:5,000 to 1:10,000 dilution is recommended. Researchers should determine optimal titers for other applications.

Storage Conditions: Store vial at 4° C prior to opening. Centrifuge product if not completely clear after standing at room temperature. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.



Purity and Specificity: This product is an IgG fraction antibody purified from antiserum by a multi-step process which includes delipidation, salt fractionation and ion exchange chromatography followed by extensive dialysis against the buffer stated above. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum. No reaction was observed with ASK-1 from mouse sources. Reactivity with the kinase from other sources has not been determined.

Immunogen: This purified antibody was prepared from rabbit serum after repeated immunizations with a KLH conjugated peptide corresponding to amino acids 76-87 of human ASK-1 protein.

Reference(s):

- Morita, K. et al. (2001) [Negative feedback regulation of ASK1 by protein phosphatase 5 \(PP5\) in response to oxidative stress](#). *EMBO J.*, **20**, 6028-6036.
- Matsuzawa, A. and Ichijo, H. (2001) [Molecular Mechanisms of the Decision between Life and Death: Regulation of Apoptosis by Apoptosis Signal-Regulating Kinase 1](#). *J. Biochem.*, **130**, 1-8.
- Zou, X. et al. (2001) [The cell cycle-regulatory CDC25A phosphatase inhibits apoptosis signal-regulating kinase 1 \(ASK1\)](#). *Mol. Cell. Biol.*, **21**, 4818-4828.
- Geleziunas, R. et al. (2001) [HIV-1 nef inhibits ASK1-dependent death signaling providing a potential mechanism for protecting the infected host cell](#). *Nature*, **410**, 834-838.
- Sayama, K. et al. (2001) [Apoptosis signal regulating kinase 1 \(ASK1\) is an intracellular inducer of keratinocyte differentiation](#). *J. Biol. Chem.*, **276**, 999-1004.
- Noguchi, K. et al. (2001) [ASK1-Signaling Promotes c-Myc Protein Stability during Apoptosis](#). *Biochem Biophys Res Commun.*, **281**, 1313-1320.
- Cho, S.-G. et al. (2001) [Glutathione s-transferase mu modulates the stress-activated signals by suppressing apoptosis signal-regulating kinase 1 \(ASK1\)](#). *J. Biol. Chem.*, **276**, 12749-12755.
- Tobiume, K. et al. (2001) [ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis](#). *EMBO reports*, **2**, 222-228.
- Takeda, K. et al. (2000) [Apoptosis Signal-regulating Kinase 1 \(ASK1\) Induces Neuronal Differentiation and Survival of PC12 Cells](#). *J. Biol. Chem.*, **275**, 9805-9813.
- Liu, H. et al. (2000) [Activation of apoptosis signal-regulating kinase 1 \(ASK1\) by tumor necrosis factor receptor-associated factor 2 \(TRAF2\) requires prior dissociation of the ASK1 inhibitor Thioredoxin](#). *Mol. Cell. Biol.*, **20**, 2198-2208.

- Kanamoto, T. et al. (2000) [Role of apoptosis signal-regulating kinase in regulation of the c-Jun N-terminal Kinase pathway and apoptosis in sympathetic neurons.](#) *Mol. Cell. Biol.*, **20**, 196-204.
- Hatai, T., Matsuzawa, A., Inoshita, S., Mochida, Y., Kuroda, T., Sakamaki, K., Kuida, K., Yonehara, S., Ichijo, H. and Takeda, K. [Execution of ASK1-induced Apoptosis by the Mitochondria-dependent Caspase Activation.](#) *J. Biol. Chem.*, **275**, 26576-26581.
- Mochida, Y. et al. (2000) [ASK1 inhibits IL-1-induced NF- \$\kappa\$ B activity through disruption of TRAF6-TAK1 interaction.](#) *J. Biol. Chem.*, **275**, 32747-32752.
- Yasuda, S. et al. (1999) [Triggering of neuronal cell death by accumulation of activated SEK1 on nuclear polyglutamine aggregations in PML bodies.](#) *Genes to Cells*, **4**, 743-756.
- Nakahara, S et al. (1999) [Induction of apoptosis signal regulating kinase 1 \(ASK1\) after spinal cord injury in rats: possible involvement of ASK1-JNK and -p38 pathways in neuronal apoptosis.](#) *J. Neuropathol. Exp. Neurol.*, **58**, 442-450.
- Nishitoh, H., et al. (1998) [ASK1 is essential for JNK/SAPK activation by TRAF2.](#) *Mol. Cell*, **2**, 389-395.
- Saitoh, M., et al. (1998) [Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase \(ASK\) 1.](#) *EMBO J.*, **17**, 2596-2606.
- Chang, HY., et al. (1998) [Activation of apoptosis signal-regulating kinase 1 \(ASK1\) by the adapter protein daxx.](#) *Science*, **281**, 1860-1863.
- Berestetskaya, Y.V., et al. (1998) [Regulation of apoptosis by alpha subunits of G12 and G13 proteins via apoptosis signal-regulating.](#) *J. Biol. Chem.*, **273**, 27816-27823.
- Wang, T.-H., et al. (1998) [Microtubule-interfering agents activate c-Jun N-terminal kinase/stress-activated protein kinase \(JNK/SAPK\) through both ras and apoptosis signal-regulating kinase \(ASK1\) pathways.](#) *J. Biol. Chem.*, **273**, 4928-4936.
- Ichijo, H., et al. (1997) [Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways.](#) *Science*, **275**, 90-94.
- Tobiume, K. et al. (1997) [Molecular cloning and characterization of the mouse apoptosis signal-regulating kinase \(ASK\) 1.](#) *Biochem. Biophys. Res. Commun.*, **239**, 905-910.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.