

Datasheet for 200-401-410**ASK1 phospho S83 Antibody****Overview**

Description:	Anti-ASK1 pS83 (RABBIT) Antibody - 200-401-410
Item No.:	200-401-410
Size:	200 µg
Applications:	ELISA, WB, IHC, IP
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	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.
Synonyms:	rabbit anti-ASK1 pS83 Antibody, Apoptosis signal regulating kinase 1 antibody, ASK 1 antibody, ASK-1 antibody, MAP/ERK kinase kinase 5 antibody, MAP3K5 antibody, MAPK/ERK kinase kinase 5 antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	MAP3K5
Reactivity:	Human
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide

Immunogen:	This purified antibody was prepared from rabbit serum after repeated immunizations with a KLH conjugated phospho-peptide corresponding to an internal region near amino acids 75-100 of human ASK-1 protein.
Purity/Specificity:	This purified antibody is directed against the phosphorylated form of ASK1 at the pS83 residues. 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.
Relevant Links:	• UniProtKB - Q99683 • NCBI - NP_005914.1 • GeneID - 4217

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IHC, IP (Based on references)
Application Note:	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. 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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5,000 - 1:10,000
WB:	1:1,000

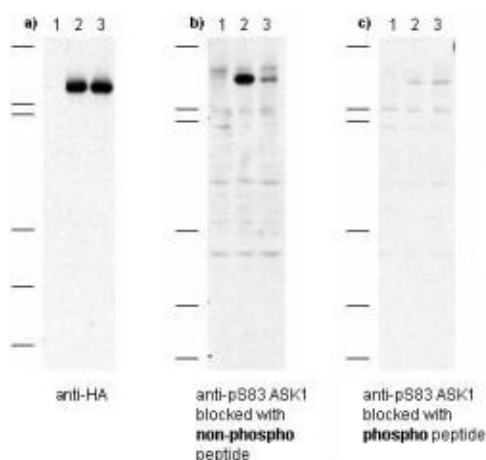
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

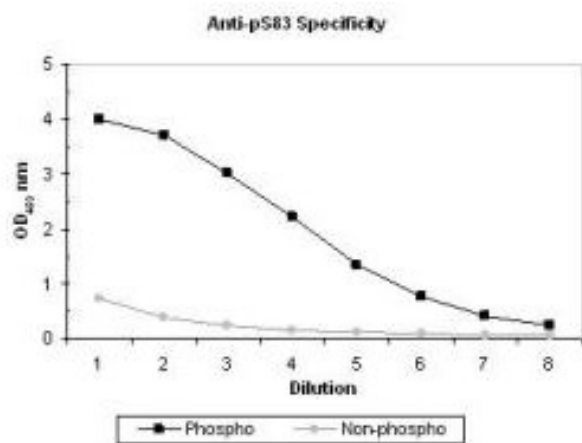
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



Western Blot

Western blot of anti-pS83 ASK1 antibodies shows specificity for phosphorylated human ASK1. Anti-pS83 (aa 76-87) antibody was tested by western blot against Cos-7 cell lysates after transient transfection 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 μ L of 1x SDS-sample buffer, heated at 96°C for 5', and vortexed for 30 sec. Samples (10 μ L 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 anti-HA antibody. This blot demonstrates both recombinant transfections express rASK1. In panel b) all samples were incubated with anti-pS83 ASK1. 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 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.



ELISA

ELISA results of purified polyclonal anti-pS83 ASK-1 antibody tested against BSA conjugates of non-phospho and phospho forms of immunizing peptide. Each well was coated with 0.1 mg of conjugate. The starting dilution of antibody was 1:1,000 and each point on the X-axis represents a 2-fold dilution. HRP conjugated Gt-a-Rabbit IgG H&L and TMB substrate were used for detection.

References

- Subramanyam M, Takahashi N, Hasegawa Y, et al. Inhibition of protein kinase Akt1 by apoptosis signal-regulating kinase-1 (ASK1) is involved in apoptotic inhibition of regulatory volume increase. *J. Biol. Chem.* (2010)
- Zou X, Tsutsui T, Ray D, et al. The cell cycle-regulatory CDC25A phosphatase inhibits apoptosis signal-regulating kinase 1. *Mol Cell Biol* (2001)
- Morita K, Saitoh M, Tobiume K, et al. Negative feedback regulation of ASK1 by protein phosphatase 5 (PP5) in response to oxidative stress. *EMBO J.* (2001)

Disclaimer

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