Product Datasheet

ASK1 phospho S83 Antibody (orb344610)

Catalog Number	orb344610
Description	ASK-1 (phospho-S83) antibody
Species/Host	Rabbit
Reactivity	Human
Conjugation	Unconjugated
Tested Applications	ELISA, WB
Immunogen	This purified antibody was prepared from rabbit serum after repeated immunizations with a KLH conjugated peptide corresponding to an internal region near amino acids 75-100 of human ASK-1 protein.
Preservatives	0.01% (w/v) Sodium Azide
Form/Appearance	Liquid (sterile filtered)
Concentration	1.0 mg/mL
Storage	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.
Note	For research use only

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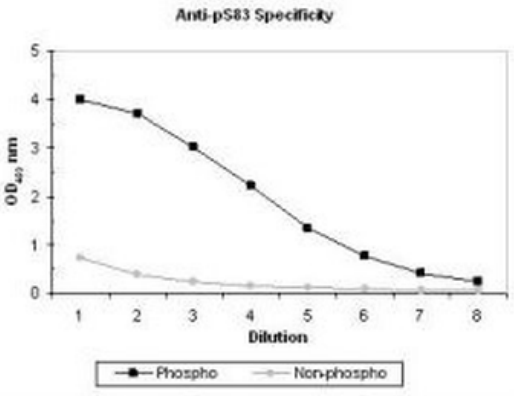
Application notes	This phospho specific polyclonal antibody reacts human pS83 ASK1 and shows minimal reactivity by western blot, ELISA and competitive ELISA with non-phosphorylated ASK1. Although not tested, this antibody is likely functional in RIA, immunohistochemistry and immunoprecipitation. For 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.
Isotype	IgG
Clonality	Polyclonal
Antibody Type	Primary Antibody
Purity	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.
Uniprot ID	**Q99683**
NCBI	**NP_005914.1**
Dilution Range	ELISA: 1:5,000 - 1:10,000, WB: 1:1,000
Expiration Date	12 months from date of receipt.



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 1:1000 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.

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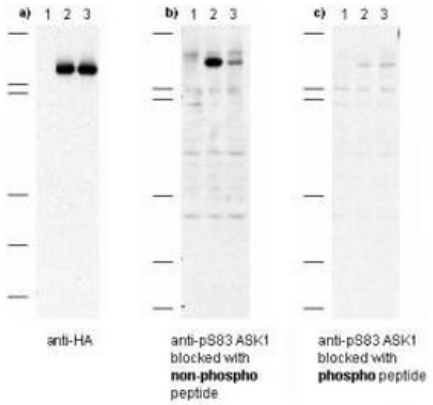

Western blot of anti-pS83 ASK1 antibodies shows specificity for phosphorylated human ASK1. Anti-pS83 (aa 76-87) antibody was tested by western blot against Cos-7 cell lysates after transient transfection with 1) vector only, 2) recombinant HA-ASK1, and 3) recombinant human HA-ASK1 where S83 was substituted with an alanine residue. Cells were lysed 24 h post-transfection in 200 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 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.

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