OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for R1172

ASK1 (MAP3K5) pSer83 Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	ELISA, WB
Recommended Dilution:	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. Recommended Dilutions: 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 1/5,000-1/10,000 dilution is recommended.
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
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.
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.
Formulation:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 with 0.01% sodium azide as preservative. State: Purified State: Liquid (sterile filtered) purified Ig fraction.
Concentration:	lot specific
Purification:	Multi-step process.
Conjugation:	Unconjugated
Storage:	Store the antibody (undiluted) at 2-8°C for one month or (in aliquots) at -20°C for longer. Dilute only prior to immediate use. Avoid repeated freezing and thawing.

Stability:	Shelf life: One year from despatch.
Gene Name:	mitogen-activated protein kinase kinase kinase 5
Database Link:	Entrez Gene 4217 Human Q99683
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:	MAPK/ERK kinase kinase 5, MAPKKK5, MAP3K5

Product images:

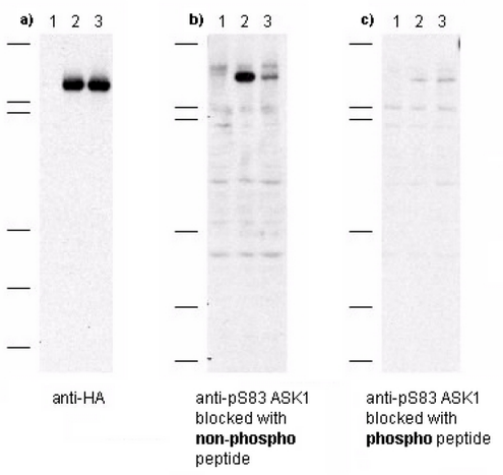

Figure 1. Immunoblot of anti-pS83 ASK1 antibodies shows specificity for phosphorylated human ASK1. Anti-pS83 (aa 76-87) antibody, generated by immunization with phospho peptide coupled to KLH, was tested by immunoblot against lysates of Cos-7 cells after transient transfection, separately, with 1) vector 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 ug/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 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 ASK1 antibody as before except the antibody was preincubated with phospho peptide prior to membrane incubation. No staining is observed after phospho peptide blocking occurs.

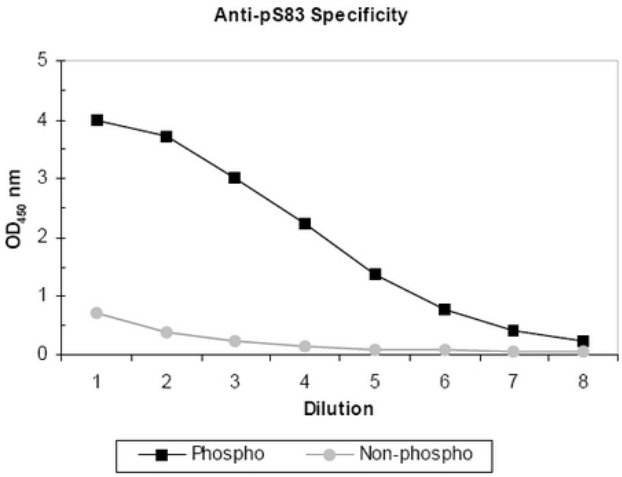

Figure 2. ELISA results of purified polyclonal anti-pS83 ASK-1 (aa 76-87) antibody tested against BSA conjugates of non-phospho and phospho forms of immunizing peptide. Each well was coated with 0.1 mg of conjugate. The starting dilution of antibody was 1:1,000 and each point on the X-axis represents a 2-fold dilution. HRP conjugated Gt-a-Rabbit IgG H&L and TMB substrate were used for detection.