

Basic Information

Product Name	Anti-ASK1/MAP3K5 Antibody	
Gene Name	MAP3K5	
Source	Rabbit	
Clonality	Polyclonal	
Isotype	IgG	
Species Reactivity	human, mouse, rat	
Tested Application	WB, IHC, ICC/IF, FCM, ELISA	
Contents	500 ug/ml antibody with PBS, 0.02% NaN ₃ , 1 mg/ml BSA and 50% glycerol.	
Immunogen	E.coli-derived human ASK1/MAP3K5 recombinant protein (Position: A178-K1112).	
Concentration	500 ug/ml	
Purification	Immunogen affinity purified.	
Observed MW	155 kDa	
Dilution Ratios	Western blot (WB): 1:500-2000 Immunohistochemistry (IHC): 1:50-400 Immunocytochemistry/Immunofluorescence (ICC/IF): 1:50-400 Flow Cytometry (Fixed): 1:50-200 Enzyme linked immunosorbent assay (ELISA): 1:100-1000 (Boiling the paraffin sections in 10mM citrate buffer, pH6.0, or PH8.0 EDTA repair liquid for 20 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user.	

Storage

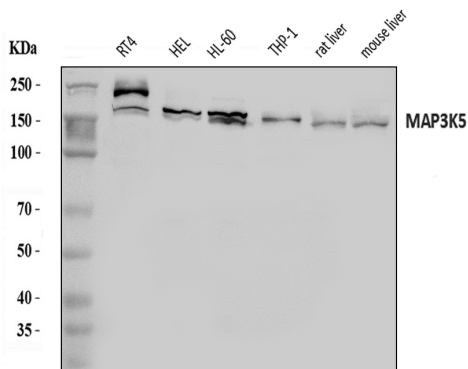
12 months from date of receipt, -20°C as supplied.

Background Information

Apoptosis signal-regulating kinase 1 (ASK1) also known as mitogen-activated protein kinase 5 (MAP3K5) is a member of MAP kinase family and as such a part of mitogen-activated protein kinase pathway. Mitogen-activated protein kinase (MAPK) signaling cascades include MAPK or extracellular signal-regulated kinase (ERK), MAPK kinase (MKK or MEK), and MAPK kinase kinase (MAPKKK or MEKK). MAPKK kinase/MEKK phosphorylates and activates its downstream protein kinase, MAPK kinase/MEK, which in turn activates MAPK. The kinases of these signaling cascades are highly conserved, and homologs exist in yeast, Drosophila, and mammalian cells. MAPKKK5 contains 1,374 amino acids with all 11 kinase subdomains. Northern blot analysis shows that MAPKKK5 transcript is abundantly expressed in human heart and pancreas. The MAPKKK5 protein phosphorylates and activates MKK4 (aliases SERK1, MAPKK4) in vitro, and activates c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK) during

transient expression in COS and 293 cells; MAPKKK5 does not activate MAPK/ERK.

Selected Validation Data



Western blot analysis of ASK1/MAP3K5 using anti-ASK1/MAP3K5 antibody (A00929-3). The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: RT4 whole cell lysates,

Lane 2: HEL whole cell lysates,

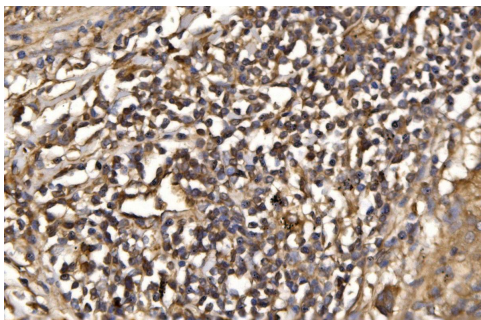
Lane 3: HL-60 whole cell lysates,

Lane 4: THP-1 whole cell lysates,

Lane 5: rat liver tissue lysates,

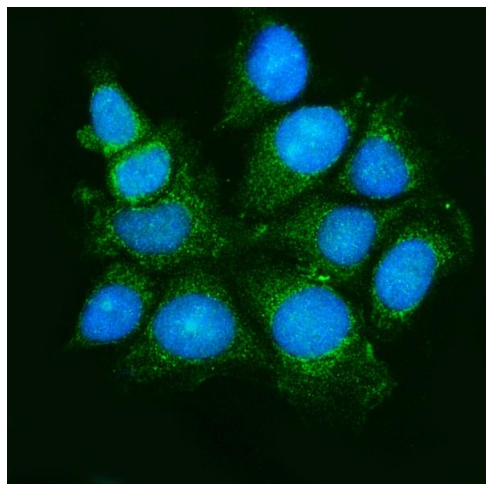
Lane 6: mouse liver tissue lysates.

After electrophoresis, proteins were transferred to a membrane. Then the membrane was incubated with rabbit anti-ASK1/MAP3K5 antigen affinity purified polyclonal antibody (A00929-3) at a dilution of 1:1000 and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054). The signal is developed using ECL Plus Western Blotting Substrate (Catalog # AR1197). A specific band was detected for ASK1/MAP3K5 at approximately 155 kDa. The expected band size for ASK1/MAP3K5 is at 155 kDa.

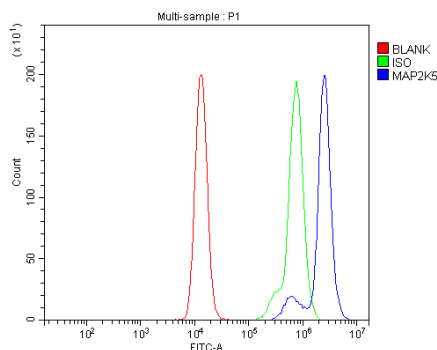


IHC analysis of ASK1/MAP3K5 using anti-ASK1/MAP3K5 antibody (A00929-3).

ASK1/MAP3K5 was detected in a paraffin-embedded section of human lung cancer tissue. Biotinylated goat anti-rabbit IgG was used as secondary antibody. The tissue section was incubated with rabbit anti-ASK1/MAP3K5 Antibody (A00929-3) at a dilution of 1:200 and developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB (Catalog # AR1027) as the chromogen.



IF analysis of ASK1/MAP3K5 using anti-ASK1/MAP3K5 antibody (A00929-3). ASK1/MAP3K5 was detected in an immunocytochemical section of MCF-7 cells. The section was incubated with rabbit anti-ASK1/MAP3K5 Antibody (A00929-3) at a dilution of 1:100. DyLight®488 Conjugated Goat Anti-Rabbit IgG (Green) (Catalog # BA1127) was used as secondary antibody. The section was counterstained with DAPI (Catalog # AR1176) (Blue).



Flow Cytometry analysis of Raji cells using anti-ASK1/MAP3K5 antibody (A00929-3).

Overlay histogram showing Raji cells stained with A00929-3 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ASK1/MAP3K5 Antibody (A00929-3) at 1:100 dilution for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG at 1:100 dilution used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.