abcam

Product datasheet

Anti-ASK1 antibody [EP553Y] ab45178





★★★★★ 3 Abreviews 33 References 7 Images

Overview

Product name Anti-ASK1 antibody [EP553Y]

Description Rabbit monoclonal [EP553Y] to ASK1

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF

Species reactivity Reacts with: Mouse, Human

Immunogen Synthetic peptide corresponding to Human ASK1 aa 1-100 (N terminal).

(Peptide available as ab224650)

Positive control IHC-P: Human lung carcinoma tisuue. WB: HeLa (ab150035), A549, and RAW 264.7 whole cell

lysates. Flow Cyt (intra): HeLa cells. ICC/IF: RAW 264.7 cells.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity - Long-term security of supply - Animal-free production

For more information see here.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this

species. Please contact us for more information.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

Purity Protein A purified

Clonality Monoclonal Clone number **EP553Y**

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab45178 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/100. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ (3)	1/1000. Detects a band of approximately 155 kDa (predicted molecular weight: 155 kDa). For unpurified use at 1/2000
IHC-P		1/50. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
ICC/IF		1/100 - 1/250.

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Function Component of a protein kinase signal transduction cascade. Phosphorylates and activates

MAP2K4 and MAP2K6, which in turn activate the JNK and p38 MAP kinases, respectively.

Overexpression induces apoptotic cell death.

Tissue specificity Abundantly expressed in heart and pancreas.

Sequence similarities Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. MAP kinase kinase

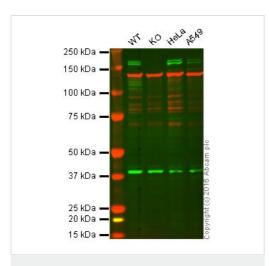
kinase subfamily.

Contains 1 protein kinase domain.

Post-translational modifications

Dephosphorylated and activated by PGAM5.

Images



Western blot - Anti-ASK1 antibody [EP553Y] (ab45178)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

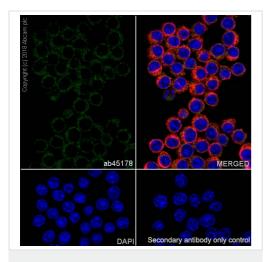
Lane 2: ASK1 knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: A549 cell lysate (20 µg)

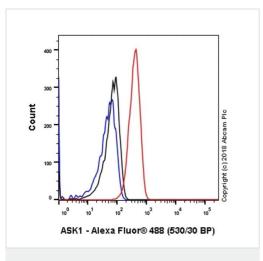
Lanes 1 - 4: Merged signal (red and green). Green - ab45178 observed at 155, 160 kDa. Red - loading control, <u>ab1240</u>, observed at 124 kDa.

Unpurified ab45178 was shown to recognize ASK1 when ASK1 knockout samples were used, along with additional cross-reactive bands. Wild-type and ASK1 knockout samples were subjected to SDS-PAGE. ab45178 and ab1240 (loading control to Vinculin) were diluted to 1/2000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



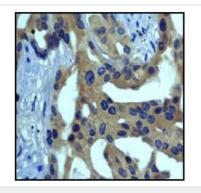
Immunocytochemistry/ Immunofluorescence - Anti-ASK1 antibody [EP553Y] (ab45178)

Immunocytochemistry/ Immunofluorescence analysis of RAW 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) cells labeling ASK1 with Purified ab45178 at 1:100 (9.9 μ g/ml). Cells were fixed in 100% Methanol. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 μ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) was used as the secondary antibody at 1:1000 (2 μ g/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-ASK1 antibody [EP553Y] (ab45178)

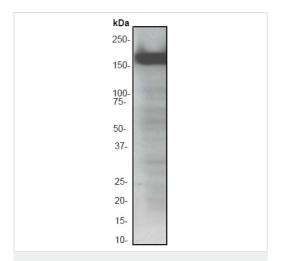
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling ASK1 with purified ab45178 at 1/100 dilution (10 μ g/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit lgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ASK1 antibody [EP553Y] (ab45178)

Unpurified ab45178 (1/10) staining human ASK1 in human lung carcinoma by immunohistochemistry using paraffin embedded tissue

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



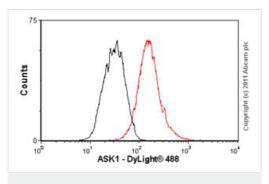
Western blot - Anti-ASK1 antibody [EP553Y] (ab45178)

Anti-ASK1 antibody [EP553Y] (ab45178) at 1/2000 dilution (Unpurified) + HeLa cell lysate at 10 μg

Secondary

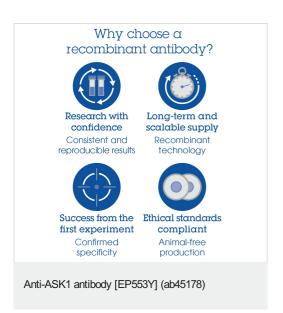
Goat anti-rabbit, HRP labelled at 1/2000 dilution

Predicted band size: 155 kDa **Observed band size:** 155 kDa



Flow Cytometry (Intracellular) - Anti-ASK1 antibody [EP553Y] (ab45178)

Overlay histogram showing HeLa cells stained with unpurified ab45178 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab45178, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was a goat anti-rabbit DyLight[®] 488 (lgG H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.



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