Anti-p38 alpha / MAPK14 Antibody [9-B9]

EM40307



Product Type: Mouse monoclonal IgG2a, primary antibodies

Species reactivity:Human, Mouse, RatApplications:WB, IF-Cell, IHC-P, FC

Molecular Wt: Predicted band size: 41 kDa

Clone number: 9-B9

Description: Mitogen-activated protein kinase 14, also called p38-α, is an enzyme that in humans is

encoded by the MAPK14 gene. The protein encoded by this gene is a member of the MAP kinase family. MAP kinases act as an integration point for multiple biochemical signals, and are involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development. This kinase is activated by various environmental stresses and proinflammatory cytokines. The activation requires its phosphorylation by MAP kinase kinases (MKKs), or its autophosphorylation triggered by the interaction of MAP3K7IP1/TAB1 protein with this kinase. The substrates of this kinase include transcription regulator ATF2, MEF2C, and MAX, cell cycle regulator CDC25B, and tumor suppressor p53, which suggest the roles of this kinase in stress-related transcription and cell cycle

regulation, as well as in genotoxic stress response.

Immunogen: Synthetic peptide within Human MAPK14 aa 1-50 / 360.

Positive control: HeLa cell lysate, Jurkat cell lysate, RAW264.7 cell lysate, PC-12 cell lysate, C6 cell lysate,

HeLa cells treated with UV for 30 minutes then recover 30 minutes, mouse stomach tissue,

rat stomach tissue, HeLa.

Subcellular location: Cytoplasm, nucleus

Database links: SwissProt: Q16539 Human | P47811 Mouse | P70618 Rat

Recommended Dilutions:

WB 1:1,000 IF-Cell 1:500 IHC-P 1:5,000 FC 1:1,000

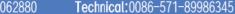
Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4° C. Store at $+4^{\circ}$ C short term (1-2 weeks). It is recommended to aliquot into

single-use upon delivery. Store at -20 °C long term.

Purity: Protein A affinity purified.

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Images

KDa xel 3 HSP90

Fig1: Western blot analysis of p38 alpha / MAPK14 on different lysates with Mouse anti-p38 alpha / MAPK14 antibody (EM40307) at 1/1,000 dilution.

Lane 1: HeLa cell lysate Lane 2: Jurkat cell lysate Lane 3: RAW264.7 cell lysate Lane 4: PC-12 cell lysate Lane 5: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 41 kDa Observed band size: 38 kDa

Exposure time: 10 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

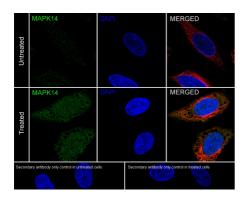


Fig2: Immunocytochemistry analysis of HeLa cells untreated / treated with UV for 30 minutes then recover 30 minutes labeling p38 alpha / MAPK14 with Mouse anti-p38 alpha / MAPK14 antibody (EM40307) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Mouse anti-p38 alpha / MAPK14 antibody (EM40307) at 1/500 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

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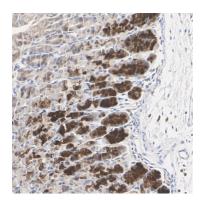


Fig3: Immunohistochemical analysis of paraffin-embedded mouse stomach tissue with Mouse anti-p38 alpha / MAPK14 antibody (EM40307) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (EM40307) at 1/5,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

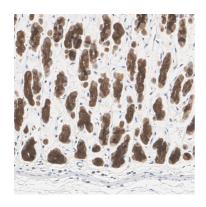


Fig4: Immunohistochemical analysis of paraffin-embedded rat stomach tissue with Mouse anti-p38 alpha / MAPK14 antibody (EM40307) at 1/5,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH $_2$ O and PBS, and then probed with the primary antibody (EM40307) at 1/5,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

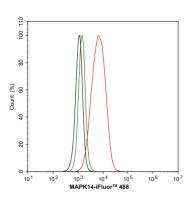


Fig5: Flow cytometric analysis of HeLa cells labeling p38 alpha / MAPK14.

Cells were fixed and permeabilized. Then stained with the primary antibody (EM40307, 1/1,000) (red) compared with Mouse IgG Isotype Control (green). After incubation of the primary antibody at +4 $^{\circ}$ C for an hour, the cells were stained with a iFluor 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

- 1. "In the cellular garden of forking paths: how p38 MAPKs signal for downstream assistance." Shi Y., Gaestel M. Biol. Chem. 383:1519-1536(2002)
- 2. "The autoimmune suppressor Gadd45alpha inhibits the T cell alternative p38 activation pathway." Salvador J.M., Mittelstadt P.R., Belova G.I., Fornace A.J. Jr., Ashwell J.D. Nat. Immunol. 6:396-402(2005)

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Applications: WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation