Anti-Phospho-p38 alpha (T180 + Y182) Antibody [PSH06-35]

HA722669

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 41 kDa
Clone number:	PSH06-35

Description: MAP (mitogen-activated protein) kinases play a significant role in many biological processes, including cell adhesion and spreading, cell differentiation and apoptosis. p38α, p38β and p38γ, also known as MAPK14, MAPK11 and MAPK12, respectively, each contain one protein kinase domain and belong to the MAP kinase family. Expressed in different areas throughout the body with common expression patterns in heart, p38 proteins use magnesium as a cofactor to catalyze the ATP-dependent phosphorylation of target proteins. Via their catalytic activity, p38α, p38β and p38γ are involved in a variety of events throughout the cell, including signal transduction pathways, cytokine production and cell proliferation and differentiation. The p38 proteins are subject to phosphoryation on Thr and Tyr residues, an event which is thought to activate the phosphorylated protein.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Thr180/Tyr182 of human p38 alpha / MAPK14.

Positive control: Jurkat cell lysate, Jurkat treated with UV for 1 hour cell lysate, NIH/3T3 treated with 25μg/mL Anisomycin for 30 minutes cell lysate, C6 treated with 25μg/mL Anisomycin for 30 minutes cell lysate, Jurkat cells treated with UV for 1 hour, NIH/3T3 cells treated with 25μg/mL Anisomycin for 30 minutes.

Subcellular location: Cytoplasm, Nucleus.

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

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:100
FC	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

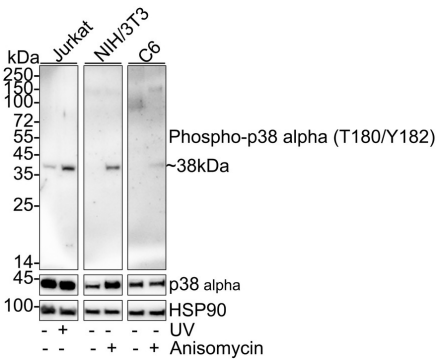

Fig1: Western blot analysis of Phospho-p38 alpha (T180 + Y182) on different lysates with Rabbit anti-Phospho-p38 alpha (T180 + Y182) antibody (HA722669) at 1/2,000 dilution and p38 alpha antibody (ET1702-65) at 1/1,000 dilution.

Lane 1: Jurkat cell lysate (20 µg/Lane)
Lane 2: Jurkat treated with UV for 1 hour cell lysate (20 µg/Lane)
Lane 3: NIH/3T3 cell lysate (20 µg/Lane)
Lane 4: NIH/3T3 treated with 25µg/mL Anisomycin for 30 minutes cell lysate (20 µg/Lane)
Lane 5: C6 cell lysate (20 µg/Lane)
Lane 6: C6 treated with 25µg/mL Anisomycin for 30 minutes cell lysate (20 µg/Lane)

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

Exposure time: 3 minutes; ECL: K1802;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722669) at 1/2,000 dilution and p38 alpha antibody (ET1702-65) at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

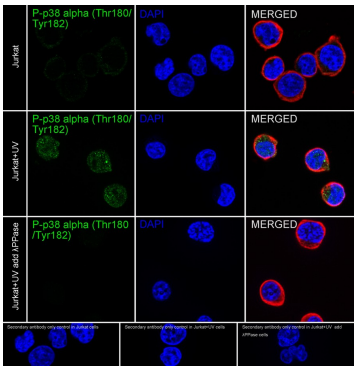

Fig2: Immunocytochemistry analysis of Jurkat cells treated with UV for 1 hour labeling Phospho-p38 alpha (T180 + Y182) with Rabbit anti-Phospho-p38 alpha (T180 + Y182) antibody (HA722669) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Rabbit anti-Phospho-p38 alpha (T180 + Y182) antibody (HA722669) at 1/50 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

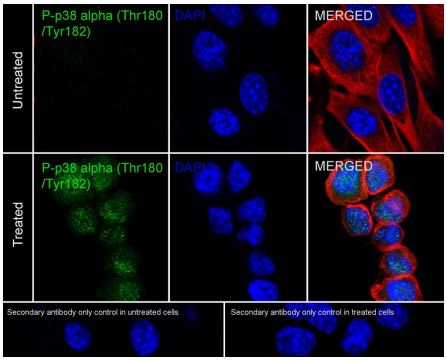

Fig3: Immunocytochemistry analysis of NIH/3T3 cells treated with 25µg/mL Anisomycin for 30 minutes labeling Phospho-p38 alpha (T180 + Y182) with Rabbit anti-Phospho-p38 alpha (T180 + Y182) antibody (HA722669) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Rabbit anti-Phospho-p38 alpha (T180 + Y182) antibody (HA722669) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

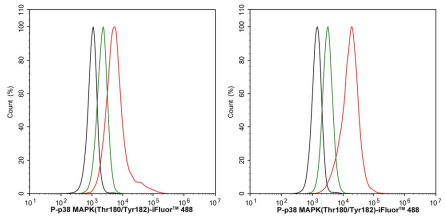

Fig4: Flow cytometric analysis of Jurkat cells untreated (left) or treated (right) with UV for 1 hour labeling Phospho-p38 alpha (T180 + Y182).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722669, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Martínez-Limón A et al. The p38 Pathway: From Biology to Cancer Therapy. Int J Mol Sci. 2020 Mar
2. Falcicchia C et al. Involvement of p38 MAPK in Synaptic Function and Dysfunction. Int J Mol Sci. 2020 Aug