Anti-CCL3 / MIP-1 alpha Antibody [PSH11-27] - BSA and Azide free

HA751406

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 10 kDa
Clone number:	PSH11-27

Description: Chemokine (C-C motif) ligand 3 (CCL3) also known as macrophage inflammatory protein 1-alpha (MIP-1-alpha) is a protein that in humans is encoded by the CCL3 gene. CCL3 is a cytokine belonging to the CC chemokine family that is involved in the acute inflammatory state in the recruitment and activation of polymorphonuclear leukocytes through binding to the receptors CCR1, CCR4 and CCR5. CCL3 produces a monophasic fever of rapid onset whose magnitude is equal to or greater than that of fevers produced with either recombinant human tumor necrosis factor or recombinant human interleukin-1. However, in contrast to these two endogenous pyrogens, the fever induced by MIP-1 is not inhibited by the cyclooxygenase inhibitor ibuprofen and CCL3 may participate in the febrile response that is not mediated through prostaglandin synthesis and clinically cannot be ablated by cyclooxygenase.

Immunogen: Recombinant protein within human CCL3 aa 1-92.

Positive control: THP-1 treated with 100nM TPA overnight then add 100ng/mL LPS for 7 hours then add 1μg/mL BFA for 3 hours cell lysate, NK-92 cell lysate, NK-92.

Subcellular location: Secreted.

Database links: SwissProt: P10147 Human

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

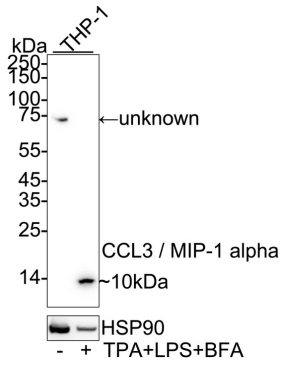

Fig1: Western blot analysis of CCL3 / MIP-1 alpha on different lysates with Rabbit anti-CCL3 / MIP-1 alpha antibody (HA751406) at 1/2,000 dilution.

Lane 1: THP-1 cell lysate
Lane 2: THP-1 treated with 100nM TPA overnight then add 100ng/mL LPS for 7 hours then add 1μg/mL BFA for 3 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 10 kDa
Observed band size: 10 kDa

Exposure time: 25 seconds; ECL: K1801;

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 (HA751406) at 1/2,000 dilution was 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.

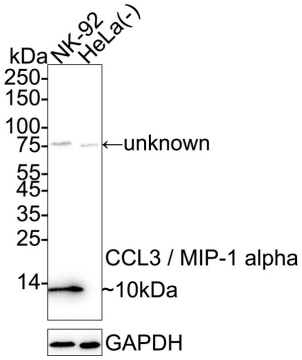

Fig2: Western blot analysis of CCL3 / MIP-1 alpha on different lysates with Rabbit anti-CCL3 / MIP-1 alpha antibody (HA751406) at 1/2,000 dilution.

Lane 1: NK-92 cell lysate
Lane 2: HeLa cell lysate (negative)

Lysates/proteins at 20 μg/Lane.

Predicted band size: 10 kDa
Observed band size: 10 kDa

Exposure time: 20 seconds; ECL: K1801;

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 (HA751406) at 1/2,000 dilution was 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.

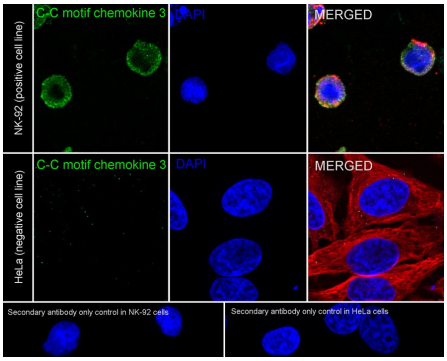

Fig3: Immunocytochemistry analysis of NK-92 (positive) and HeLa (negative) labeling CCL3 / MIP-1 alpha with Rabbit anti-CCL3 / MIP-1 alpha antibody (HA751406) at 1/100 dilution.

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 Rabbit anti-CCL3 / MIP-1 alpha antibody (HA751406) 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 (HA601187, 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.

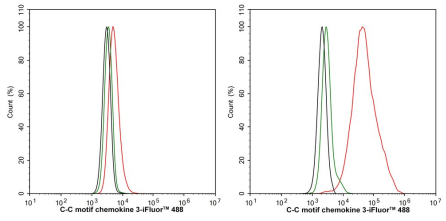

Fig4: Flow cytometric analysis of HeLa (left, negative) and NK-92 (right, positive) cells labeling CCL3 / MIP-1 alpha.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751406, 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. Sheng D et al. Ccl3 enhances docetaxel chemosensitivity in breast cancer by triggering proinflammatory macrophage polarization. J Immunother Cancer. 2022 May
2. Yang YL et al. The Role of CCL3 in the Pathogenesis of Rheumatoid Arthritis. Rheumatol Ther. 2023 Aug