Anti-CCL3 / MIP-1 alpha Antibody [PSH11-28]

HA723310

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 10 kDa
Clone number:	PSH11-28

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, RAW264.7 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, RAW264.7 cells treated with 100nM TPA overnight then add 100ng/mL LPS for 7 hours, human hodgkin lymphoma tissue, human spleen tissue.

Subcellular location: Secreted.

Database links: SwissProt: P10147 Human | P10855 Mouse

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:2,000
IHC-P	1:200

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

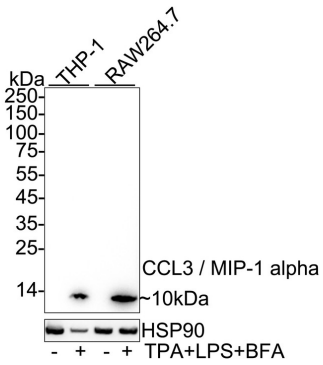

Fig1: Western blot analysis of CCL3 / MIP-1 alpha on different lysates with Rabbit anti-CCL3 / MIP-1 alpha antibody (HA723310) at 1/2,000 dilution.

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
Lane 3: RAW264.7 cell lysate
Lane 4: RAW264.7 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: 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 (HA723310) 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.

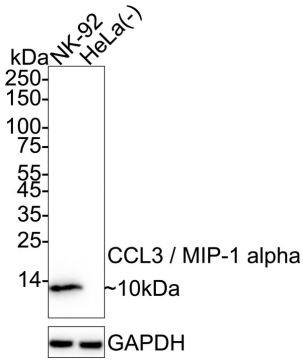

Fig2: Western blot analysis of CCL3 / MIP-1 alpha on different lysates with Rabbit anti-CCL3 / MIP-1 alpha antibody (HA723310) at 1/2,000 dilution.

Lane 1: NK-92 cell lysate (20 μg/Lane)
Lane 2: HeLa cell lysate (negative) (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 (HA723310) 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.

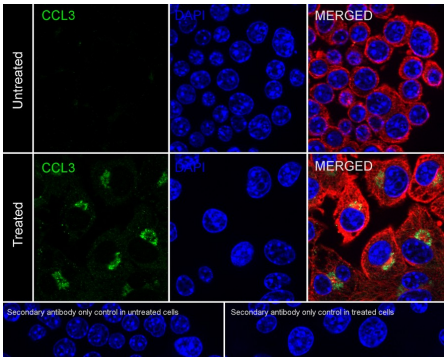

Fig3: Immunocytochemistry analysis of RAW264.7 cells untreated / treated with 100nM TPA overnight then add 100ng/mL LPS for 7 hours labeling CCL3 / MIP-1 alpha with Rabbit anti-CCL3 / MIP-1 alpha antibody (HA723310) at 1/2,000 dilution.

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 (HA723310) at 1/2,000 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.

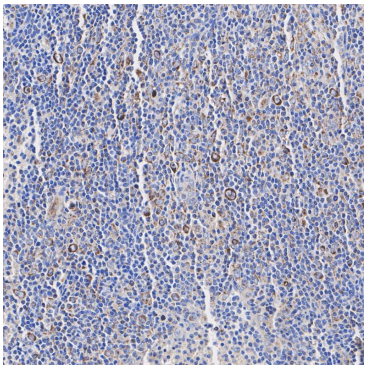

Fig4: Immunohistochemical analysis of paraffin-embedded human hodgkin lymphoma tissue with Rabbit anti-CCL3 / MIP-1 alpha antibody (HA723310) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (HA723310) at 1/200 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.

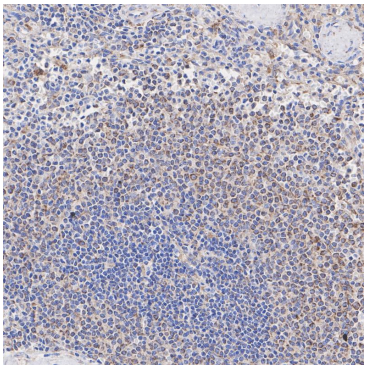

Fig5: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-CCL3 / MIP-1 alpha antibody (HA723310) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (HA723310) at 1/200 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.

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