

Anti-CXCL5 Antibody [JE53-78]

HA722561



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 12 kDa
Clone number:	JE53-78

Description: The protein encoded by this gene, CXCL5 is a small cytokine belonging to the CXC chemokine family that is also known as epithelial-derived neutrophil-activating peptide 78 (ENA-78). It is produced following stimulation of cells with the inflammatory cytokines interleukin-1 or tumor necrosis factor- α . Expression of CXCL5 has also been observed in eosinophils, and can be inhibited with the type II interferon IFN- γ . This chemokine stimulates the chemotaxis of neutrophils possessing angiogenic properties. It elicits these effects by interacting with the cell surface chemokine receptor CXCR2. The gene for CXCL5 has four exons and is located on human chromosome 4 amongst several other CXC chemokine genes. CXCL5 has been implicated in connective tissue remodelling. CXCL5 has been also described to regulate neutrophil homeostasis.

Immunogen: Synthetic peptide within human CXCL5 aa 65-114 / 114.

Positive control: A549 starved overnight treated with 10ng/mL TNF- α and 10nM PMA for 24 hours then add 300ng/mL BFA for 3 hours cell lysate, A549 cells starved overnight treated with 10ng/mL TNF- α and 10nM PMA for 24 hours then add 300ng/mL BFA for 3 hours.

Subcellular location: Secreted.

Database links: SwissProt: P42830 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:500
FC	1:1,000
IP	1-2 μ g/sample

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

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C long term.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

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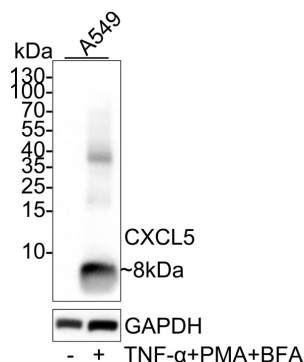
Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

Fig1: Western blot analysis of CXCL5 on different lysates with Rabbit anti-CXCL5 antibody (HA722561) at 1/1,000 dilution.

Lane 1: A549 cell lysate

Lane 2: A549 starved overnight treated with 10ng/mL TNF-alpha and 10nM PMA for 24 hours then add 300ng/mL BFA for 3 hours cell lysate



Lysates/proteins at 20 µg/Lane.

Predicted band size: 12 kDa

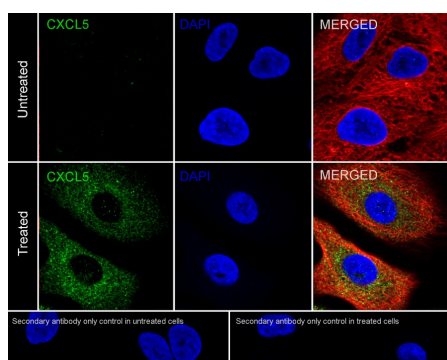
Observed band size: 8 kDa

Exposure time: 16 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 (HA722561) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C 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 A549 cells starved overnight treated with 10ng/mL TNF-alpha and 10nM PMA for 24 hours then add 300ng/mL BFA for 3 hours labeling CXCL5 with Rabbit anti-CXCL5 antibody (HA722561) at 1/500 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-CXCL5 antibody (HA722561) at 1/500 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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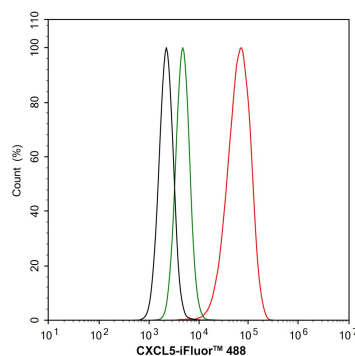


Fig3: Flow cytometric analysis of HeLa cells starved overnight treated with 10ng/mL TNF-alpha and 10nM PMA for 24 hours then add 300ng/mL BFA for 3 hours labeling CXCL5.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722561, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

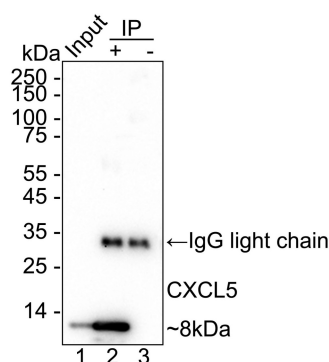


Fig4: CXCL5 was immunoprecipitated from 0.2 mg A549 starved overnight treated with 10ng/mL TNF-alpha and 10nM PMA for 24 hours then add 300ng/mL BFA for 3 hours cell lysate with HA722561 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA722561 at 1/1,000 dilution. Anti-Rabbit IgG for IP, AlpSdAbs® VHH(HRP) (025-100-005) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: A549 starved overnight treated with 10ng/mL TNF-alpha and 10nM PMA for 24 hours then add 300ng/mL BFA for 3 hours cell lysate (input)

Lane 2: HA722561 IP in A549 starved overnight treated with 10ng/mL TNF-alpha and 10nM PMA for 24 hours then add 300ng/mL BFA for 3 hours cell lysate

Lane 3: Rabbit IgG instead of HA722561 in A549 starved overnight treated with 10ng/mL TNF-alpha and 10nM PMA for 24 hours then add 300ng/mL BFA for 3 hours cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST

Exposure time: 38 seconds; ECL: K1801

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

Background References

1. Cao Q et al. Astrocytic CXCL5 hinders microglial phagocytosis of myelin debris and aggravates white matter injury in chronic cerebral ischemia. *J Neuroinflammation*. 2023 May
2. Chen C et al. CXCL5 suppression recovers neovascularization and accelerates wound healing in diabetes mellitus. *Cardiovasc Diabetol*. 2023 Jul

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