

Active Chemokine C-X3-C-Motif Ligand 1 (CX3CL1) Instruction Manual

SBPA023Hu02

Homo sapiens (Human)

Buffer Formulation

20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% SKL, 5% Trehalose and Proclin300.

Traits

Freeze-dried powder

Purity

> 90%

Isoelectric Point

6.1

Applications

Cell culture; Activity Assays.

ACTIVITY TEST

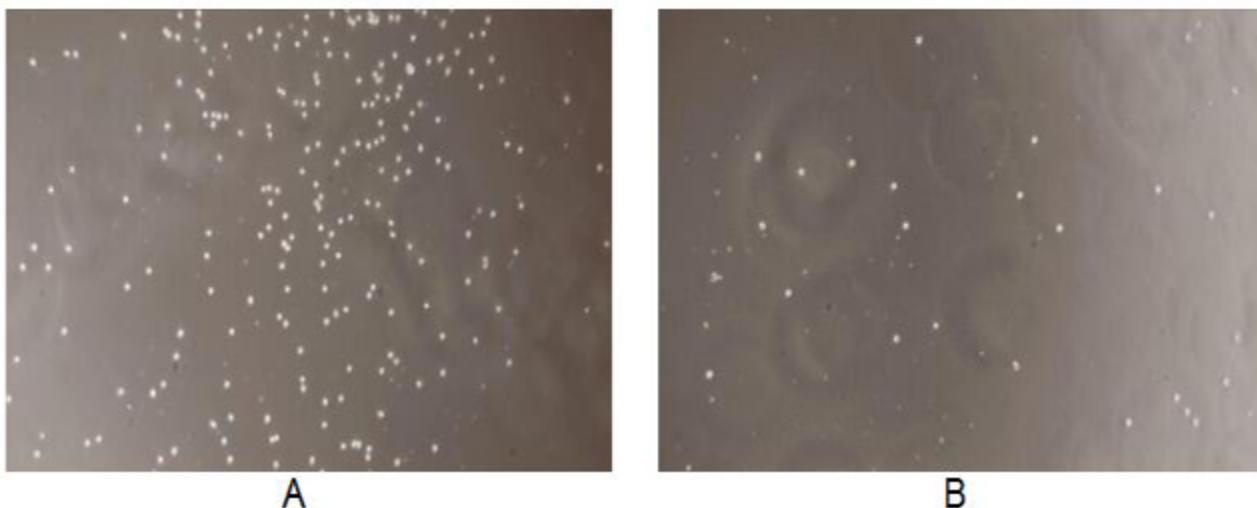


Figure. The chemotactic effect of CX3CL1 on THP-1 cells.

Chemokine C-X3-C-Motif Ligand 1 (CX3CL1) also known as fractalkine is a large cytokine protein of 373 amino acids, it contains multiple domains and is the only known member of the CX3C chemokine family. Soluble CX3CL1 potently chemoattracts T cells and monocytes, while the cell-bound chemokine promotes strong adhesion of leukocytes to activated endothelial cells, where it is primarily expressed. Thus, chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the chemotactic effect of CX3CL1 on the human monocytic cell line THP-1. Briefly, THP-1 cells were seeded into the upper chambers (150uL cell suspension, 106 cells/mL in RPMI 1640 with FBS free)

and SLC (1ng/mL, 10ng/mL, 100ng/mL and 1000ng/mL diluted separately in serum free RPMI 1640) was added in lower chamber with a polycarbonate filter (8um pore size) used to separate the two compartments. After incubation at 37°C with 5% CO₂ for 1h, the filter was removed, then cells in low chamber were observed by inverted microscope at low magnification (×100) and the number of migrated cells were counted at high magnification (×400) randomly (five fields for each filter). Result shows CX3CL1 is able to induce migration of THP-1 cells. The migrated Jurkat cells in low chamber at low magnification (×100) were shown in Figure 1. Five fields of each chamber were randomly chosen, and the migrated cells were counted at high magnification (×400). Statistical results were shown in Figure 2. The optimum chemotaxis of CX3CL1 occurs at 1-1000ng/mL.

(A) THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 with 10ng/mL CX3CL1 was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 1h;

(B) THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 without CX3CL1 was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 1h.

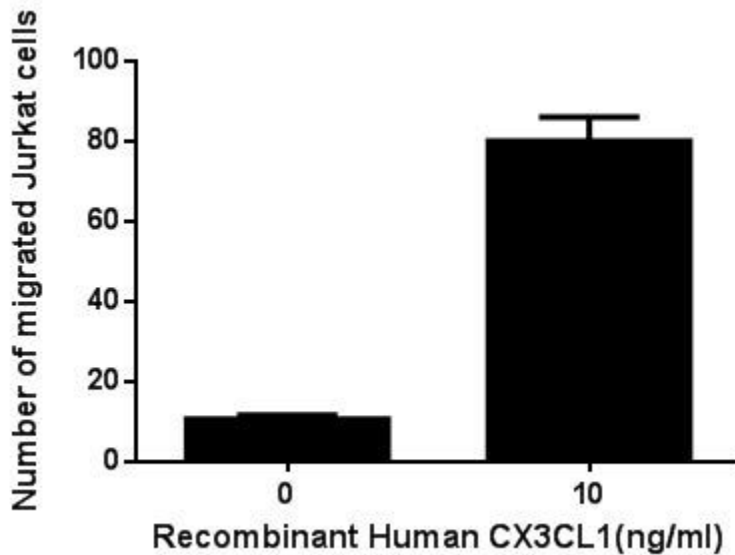


Figure. The chemotactic effect of CX3CL1 on THP-1 cells.

USAGE

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

STORAGE

Avoid repeated freeze/thaw cycles. Store at 2-8°C for one month. Aliquot and store at -80°C for 12 months.

STABILITY

The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

Image

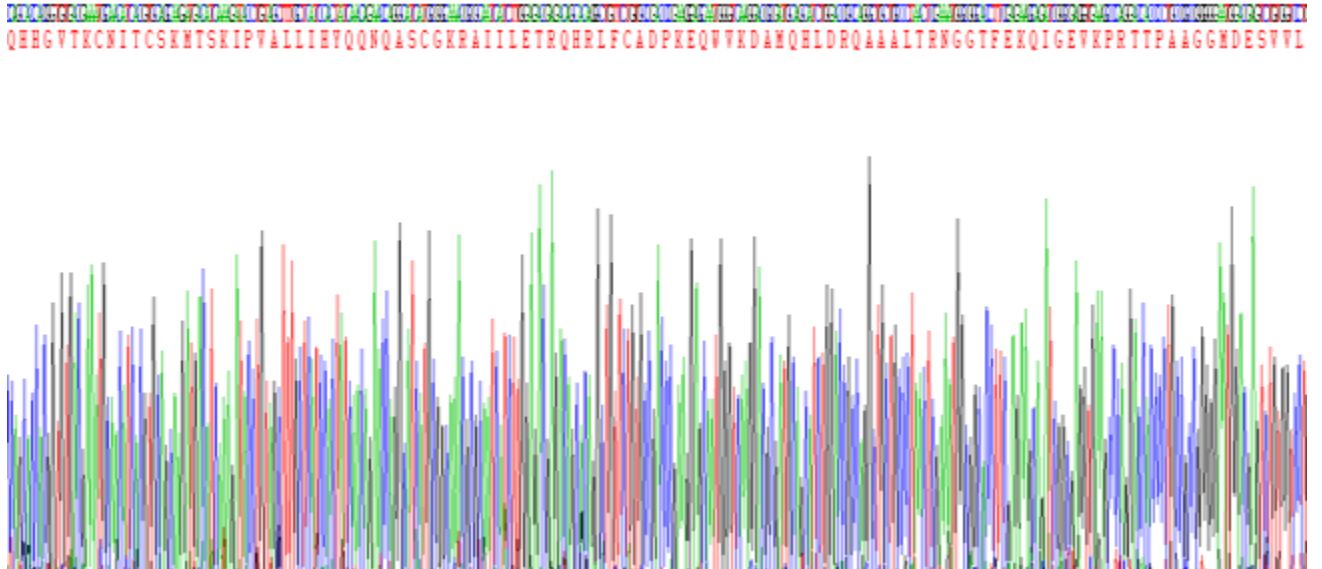


Figure. SDS-PAGE

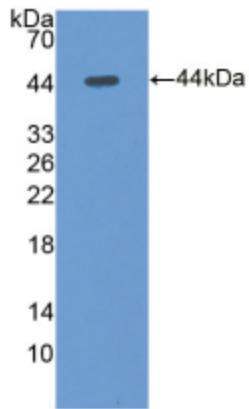


Figure. Western Blot

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.