

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

SBPA023Hu01

Homo sapiens (Human)

Buffer Formulation	PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.
Traits	Freeze-dried powder
Purity	> 95%
Isoelectric Point	5.0
Applications	Cell culture; Activity Assays.

ACTIVITY TEST

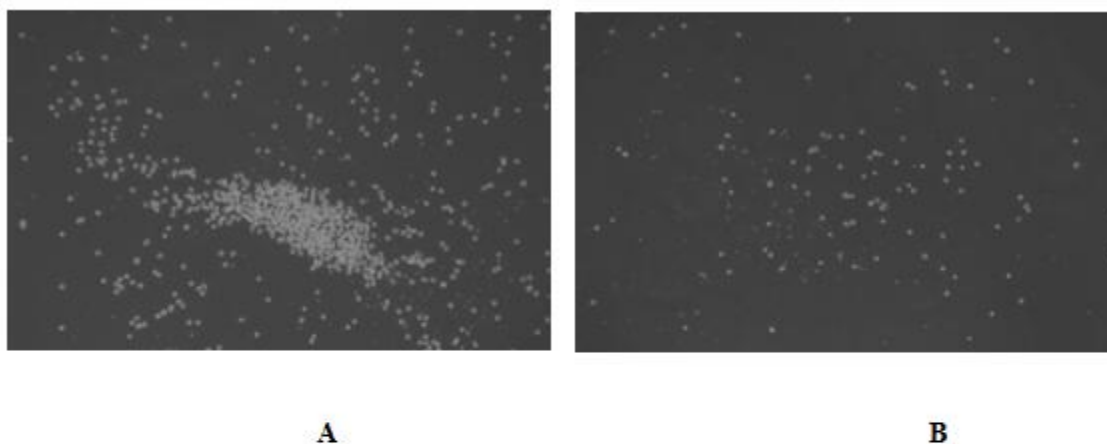


Figure 1. The chemotactic effect of CX3CL1 on THP-1 cells

(A) THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 with 0.00001 ng/ml CX3CL1 was added in lower chamber, then cells in lower chamber were observed at low magnification($\times 10$) 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($\times 10$) after incubation for 1h.

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 CX3CL1 (0.1ng/ml, 0.01ng/ml, 0.001ng/ml, 0.0001 and 0.00001ng/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 (×10) and the number of migrated cells were counted using Fluorescence Activating Cell Sorter. Result shows CX3CL1 is able to induce migration of THP-1 cells. The migrated THP-1 cells in low chamber at low magnification (×10) were shown in Figure 1. Statistical results of FACS were shown in Figure 2. The optimum chemotaxis of CX3CL1 occurs at 0.001-0.00001ng/ml.

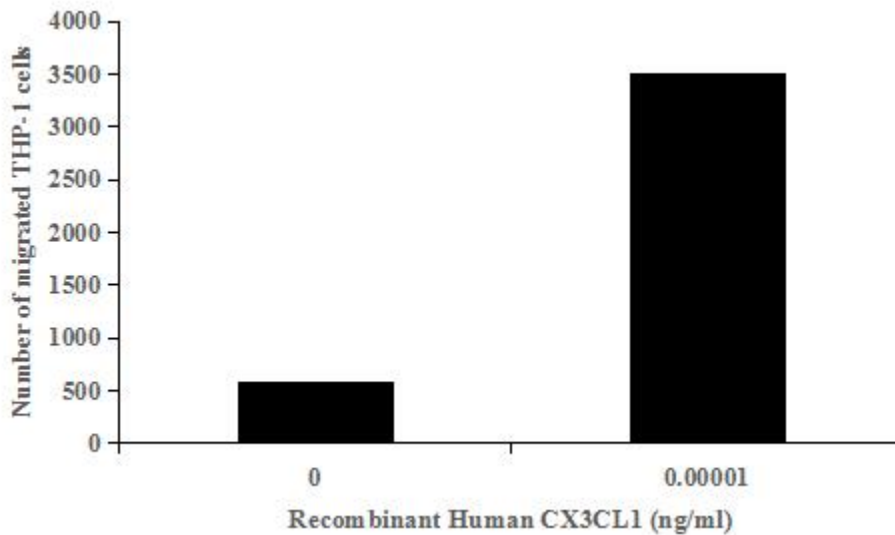


Figure 2. The chemotactic effect of CX3CL1 on THP-1 cells

USAGE

Reconstitute in 10mM PBS (pH7.4) 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

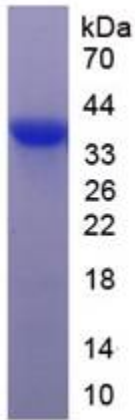


Figure. SDS-PAGE

[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.