APA040Hu01 100μg
Active Chemokine C-X3-C-Motif Ligand 1 (CX3CL1)
Organism Species: *Homo sapiens (Human)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Trp81~Ala336
Tags: N-terminal His-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.0
Predicted Molecular Mass: 28.5kDa
Accurate Molecular Mass: 39kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: 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.

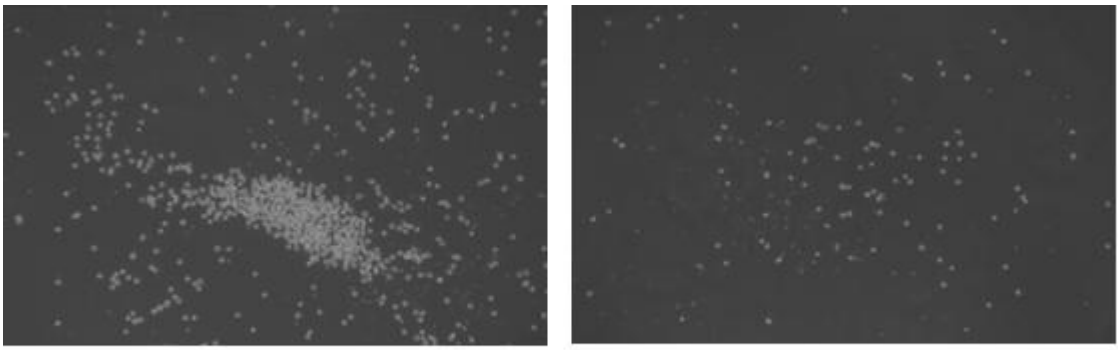
[SEQUENCE]

WVKDAMQHLD RQAAALTRNG
GTFEKQIGEV KPRTTPAAGG MDESVVLEPE ATGESSSLEP TPSSQEAQRA
LGTSPELPTG VTGSSGTRLP PTPKAQDGGP VGTELFRVPP VSTAATWQSS
APHQPGPSLW AEAKTSEAPS TQDPSTQAST ASSPAPEENA PSEGQRVWGQ
GQSPRPENSL EREEMGPVPA HTDAFQDWGP GSMAHVSVVP VSSEGTPSRE
PVASGSWTPK AEEPIHATMD PQRLGVLITP VPDAQA

[ACTIVITY]

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,10^6 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℃ with 5% CO_2 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.



A B

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(×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(×10) after incubation for 1h.

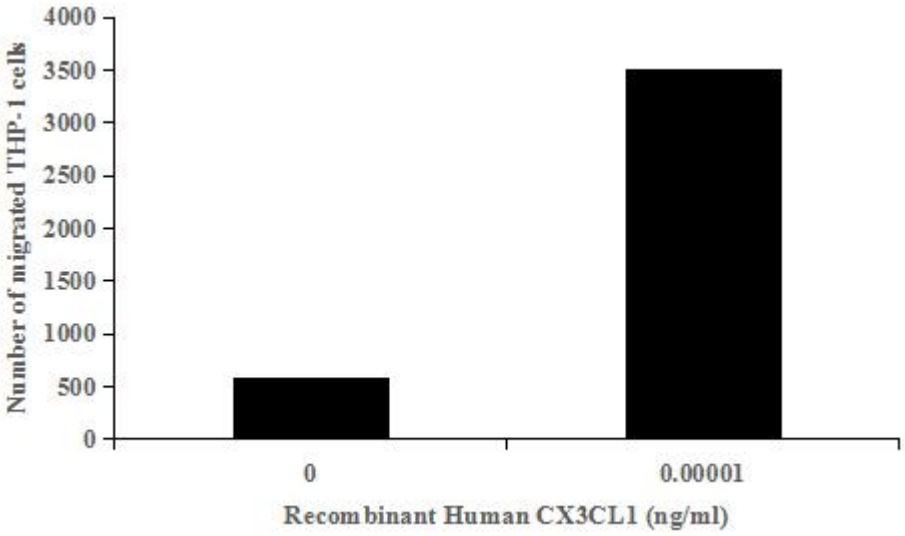


Figure 2.The chemotactic effect of CX3CL1 on THP-1 cells

[IDENTIFICATION]

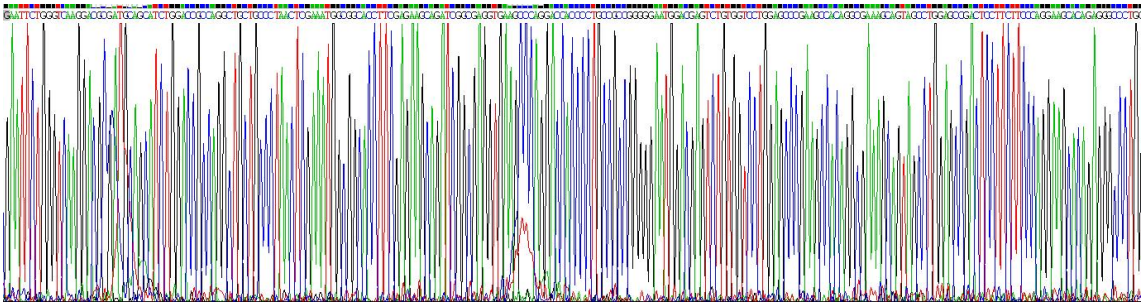


Figure 3. Gene Sequencing (extract)

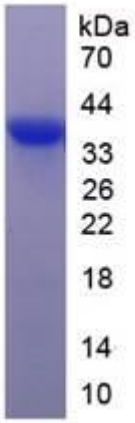


Figure 4. SDS-PAGE

Sample: Active recombinant CX3CL1, Human

[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.