APA040Hu02 100μg
Active C-X3-C-M Chemokine otif Ligand 1 (CX3CL1)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Gln25~Pro332
Tags: N-terminal His-tag
Purity: >92%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 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: 6.1
Predicted Molecular Mass: 36.3kDa
Accurate Molecular Mass: 44kDa 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 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8ºC for one month.

Aliquot and store at -80ºC 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 37ºC 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]

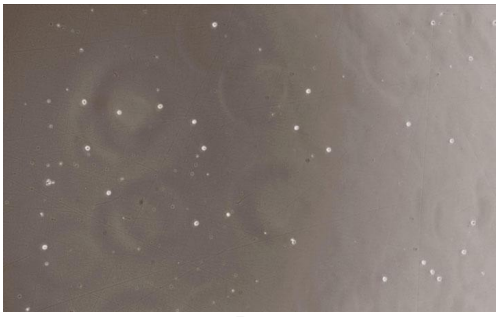
QHHGVT KCNITCSKMT SKIPVALLIH
YQQNQASCGK RAIILETRQH RLFCADPKEQ WVKDAMQHLD RQAAALTRNG
GTFEKQIGEV KPRTTPAAGG MDESVVLEPE ATGESSSLEP TPSSQEAQRA
LGTSPELPTG VTGSSGTRLP PTPKAQDGGP VGTELFRVPP VSTAATWQSS
APHQPGPSLW AEAKTSEAPS TQDPSTQAST ASSPAPEENA PSEGQRVWGQ
GQSPRPENSL EREEMGPVPA HTDAFQDWGP GSMAHVSVVP VSSEGTPSRE
PVASGSWTPK AEEPIHATMD PQRLGVLITP VP

[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 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℃ with 5% CO_2 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



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 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.

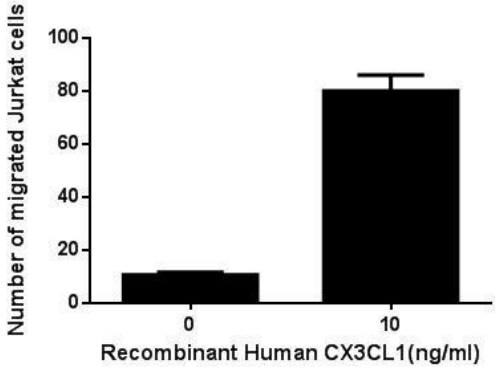


Figure 2. The chemotactic effect of CX3CL1 on THP-1 cells.

[IDENTIFICATION]

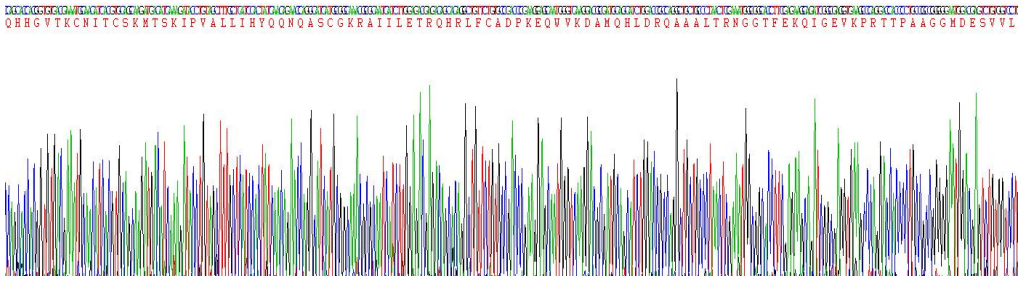


Figure 3. Gene Sequencing (extract)

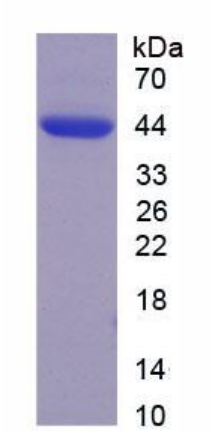


Figure 4. SDS-PAGE

Sample: Active recombinant CX3CL1, Human

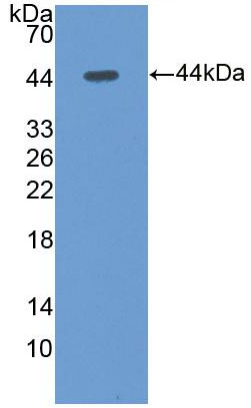


Figure 5. Western Blot

Sample: Recombinant CX3CL1, Human;

Antibody: Rabbit Anti-Human CX3CL1 Ab (PAA040Hu02)

[IMPORTANT NOTE]

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