

APA216Hu51 100µg

Active Monocyte Chemotactic Protein 4 (MCP4)

Organism Species: Homo sapiens (Human)

Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.

Host: Yeast

Residues: Phe17~Thr98

Tags: N-terminal His-tag

Purity: >95%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% sarcosyl, 5% trehalose, and Proclin300.

Applications: Cell culture; Activity Assays; In vivo assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 10.0

Predicted Molecular Mass: 10.7kDa

Accurate Molecular Mass: 13kDa as determined by SDS-PAGE reducing conditions.

[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 2-8°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]

FNPQ GLAQPDALNV PSTCCFTFSS KKISLQRLKS
YVITTSRCPQ KAVIFRTRLG KEICADPKEK WVQNYMKHLG RKAHTLKT

[ACTIVITY]

CCL13 (C-C motif chemokine 13) is a chemotactic factor that attracts monocytes, lymphocytes, basophils and eosinophils, which belongs to the CC chemokine subfamily. It has been reported that CCL13 can induce chemotactic migration of THP-1 cells. Therefore, chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the chemotactic effect of CCL13 on the human monocytic cell line THP-1. Briefly, THP-1 cells were seeded into the upper chambers (100µL cell suspension, 10⁶ cells/mL in RPMI 1640 with 0.5% FBS) and CCL13 (50ng/mL and 100ng/mL diluted separately in serum free RPMI 1640) was added in lower chamber with a polycarbonate filter (8µm pore size) used to separate the two compartments. After incubation at 37°C with 5% CO₂ for 3h, 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: CCL13 is able to induce migration of THP-1 cells. The migrated THP-1 cells in low chamber at low magnification (×100) were shown in Figure 1. Five fields of each chamber were randomly chosen to count the migrated cells at high magnification (×400) and the statistical data was shown in Figure 2.

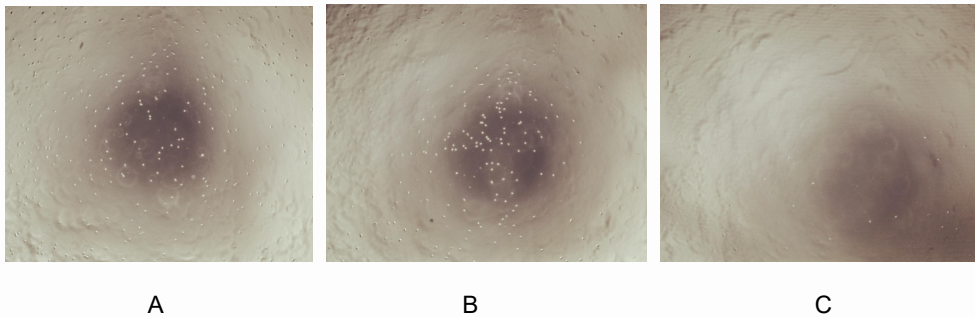


Figure 1. The chemotactic effect of CCL13 on THP-1 cells.

(A) THP-1 cells were seeded into the upper chambers and 50ng/mL CCL13 was added in lower chamber, then cells in lower chamber were observed at low magnification ($\times 100$) after incubation for 3h;

(B) THP-1 cells were seeded into the upper chambers and 100ng/mL CCL13 was added in lower chamber, then cells in lower chamber were observed at low magnification ($\times 100$) after incubation for 3h;

(C) THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 without CCL13 was added in lower chamber, then cells in lower chamber were observed at low magnification ($\times 100$) after incubation for 3h.

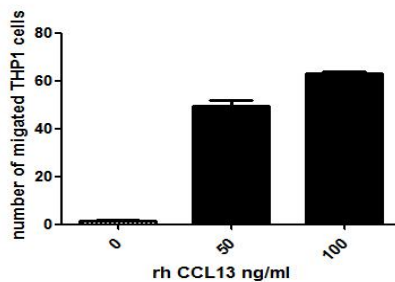


Figure 2. The chemotactic effect of CCL13 on THP-1 cells.

[IDENTIFICATION]

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TTGACDCCGGGACTGCTGAGGAGTGCCTAGAGTGCATCTCTGCTGCTGATTTGGAGTAAAGAGTCTCTTGAGAGCTGAGAGCTATGTGATCCCGCGAGGTGCGGAGAGGCTGATCTTCAAGACAACTGGGAGGAGTCTGCTGACCGAAGGAGAGTGCCTGAT  
F N P Q G L A Q P D A I N V P S T C C F T F S S K R I S L Q R L K S Y V I T T S R C P Q K A V I F R T K L G K E I C A D P K E K V V Q N
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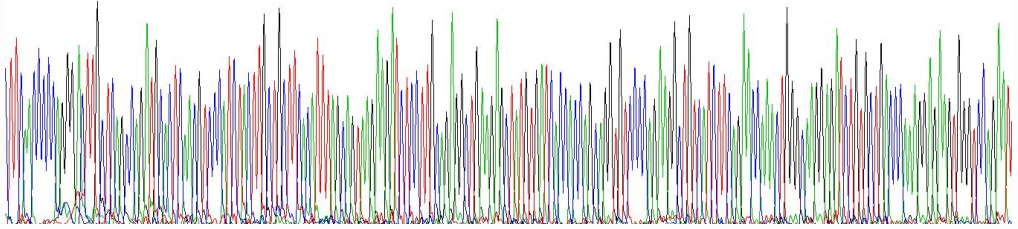


Figure 3. Gene Sequencing (extract)

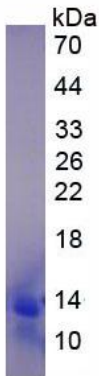


Figure 4. SDS-PAGE

Sample: Active recombinant MCP4, Human

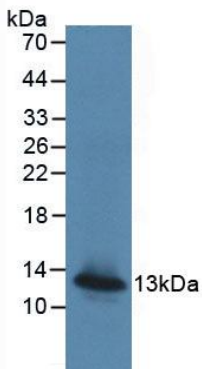


Figure 5. Western Blot

Sample: Recombinant MCP4, Human;

Antibody: Rabbit Anti-Human MCP4 Ab (PAA216Hu07)