

APA087Ra01 100µg

Active Monocyte Chemotactic Protein 1 (MCP1)

Organism Species: *Rattus norvegicus* (Rat)

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: Gln24~Asn148

Tags: N-terminal His-tag

Purity: >98%

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: 9.1

Predicted Molecular Mass: 15.4kDa

Accurate Molecular Mass: 19kDa as determined by SDS-PAGE reducing conditions.

[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]

QPDAVNA PLTCCYSFTG KMIPMSRLEN YKRITSSRCP KEAVVFVTKL
KREICADPNK EWWQKYIRKL DQNQVRSETT VFYKIASTLR
TSAPLNVNLT HKSEANASTL FSTTTSSTSV EVTSMTEN

[ACTIVITY]

Monocyte Chemotactic Protein 1 (MCP1), also known as C-C motif chemokine 2(CCL2), is a member of the β (C-C) subfamily of chemokines that is a chemoattractant for monocytes and basophils. Rat CCL2 is secreted as a 14 kDa glycoprotein monomer but noncovalent dimers probably occur. CCL2 is best known as a chemotactic agent for mononuclear cells. Thus, chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the chemotactic effect of recombinant rat MCP1 on the THP-1 cell line. Briefly, THP-1 cells were seeded into the upper chambers (150 μ L cell suspension, 10^6 cells/mL in RPMI 1640 with FBS free) and MCP1 (0.01 ng/mL, 0.1 ng/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL and 1000 ng/mL diluted separately in serum free RPMI 1640) was added in lower chamber with a polycarbonate filter (8 μ m pore size) used to separate the two compartments. After incubation at 37 °C with 5% CO₂ for 2h, the filter was removed, then cells in low chamber were observed by inverted microscope at low magnification (\times 100) and the number of migrated cells were counted at high magnification (\times 400) randomly (five fields for each filter). Result shows MCP1 is able to induce migration of THP-1 cells. The migrated THP-1 cells in low chamber at low magnification (\times 100) were shown in Figure 1.

Five fields of each chamber were randomly chosen, and the migrated cells were counted at high magnification ($\times 400$). Statistical results were shown in Figure 2. The optimum chemotaxis of recombinant rat MCP1 occurs at 0.01-0.1 ng/mL.

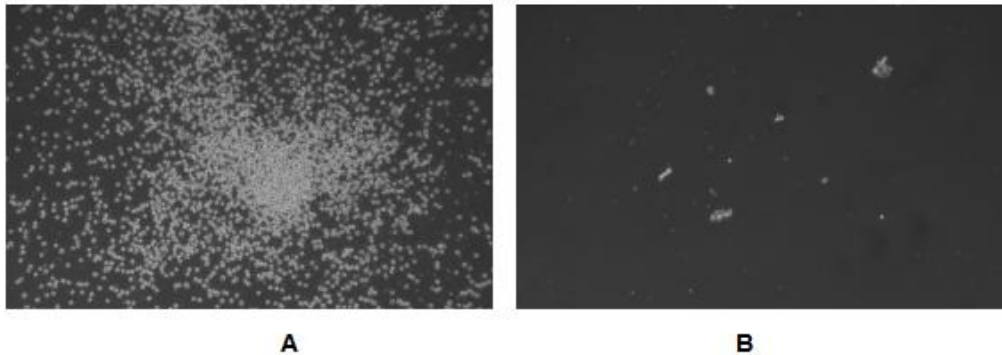


Figure 1. The chemotactic effect of recombinant rat MCP1 on THP-1 cells

- (A) THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 with 0.1 ng/mL MCP1 was added in lower chamber, then cells in lower chamber were observed at low magnification ($\times 100$) after incubation for 2h;
- (B) THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 without MCP1 was added in lower chamber, then cells in lower chamber were observed at low magnification ($\times 100$) after incubation for 2h.

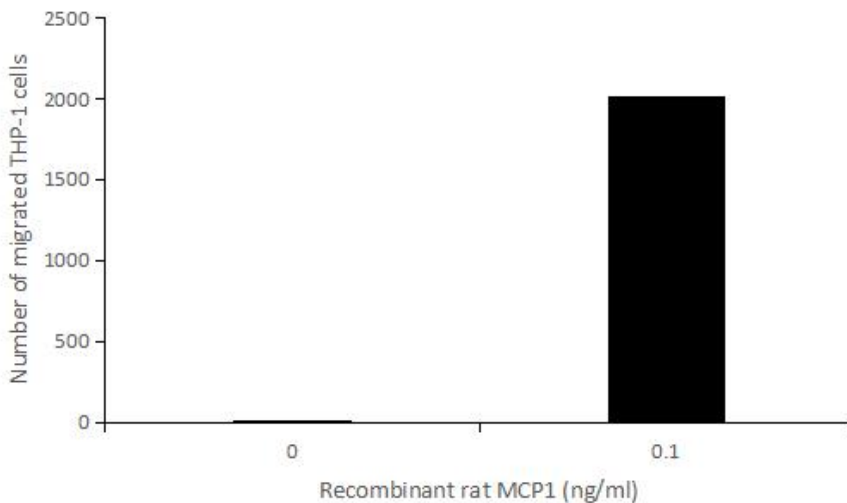


Figure 2. The chemotactic effect of recombinant rat MCP1 on THP-1 cells

[IDENTIFICATION]

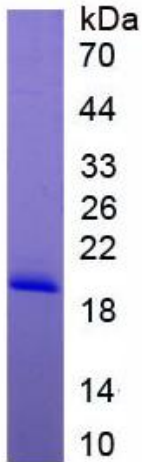


Figure 3. SDS-PAGE

Sample: Active recombinant MCP1, Rat

[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.