APA087Hu01 10μg
Active Monocyte Chemotactic Protein 1 (MCP1)
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: Gln24~Thr99

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 0.01% skl, 5%Trehalose.

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: 9.4

Predicted Molecular Mass: 9.5kDa

Accurate Molecular Mass: 15&17kDa 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 2-8°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]

QPDAINA PVTCCYNFTN RKISVQRLAS
YRRITSSKCP KEAVIFKTIV AKEICADPKQ KWVQDSMDHL DKQTQTPKT

[ACTIVITY]

MCP-1 (monocyte chemoattractant protein 1), also known as CCL2 (C-C motif chemokine 2), is a small cytokine that belongs to the CC chemokine family. MCP-1 has been described as a chemoattractant for monocytes and proven to be able to induce chemotactic migration of THP-1 cells. Therefore, chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the chemotactic effect of MCP-1 on the human monocytic cell line THP-1. Briefly, THP-1 cells were seeded into the upper chambers (100uL cell suspension, 10^6 cells/mL in RPMI 1640 with 0.5% FBS) and MCP-1 (25ng/mL and 50ng/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% CO_2 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: MCP-1 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, and the migrated cells were counted at high magnification (×400). Statistical result were shown in Figure 2. The optimum chemotactic concentration of MCP-1 is about 25ng/mL, which is in accordance with references.

A B C

Figure 1. The chemotactic effect of MCP-1 on THP1 cells.

(A) THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 with 50ng/mL MCP-1 was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 3h;

(B) THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 with 25ng/mL MCP-1 was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 3h;

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

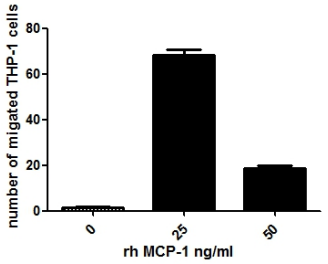

Figure 2. The chemotactic effect of MCP-1 on THP-1 cells

[IDENTIFICATION]

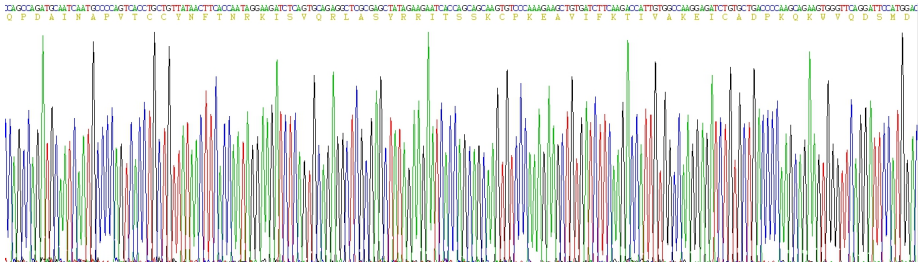

Figure 3. Gene Sequencing (extract)

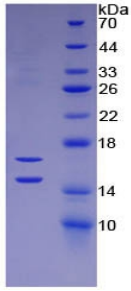

Figure 4. SDS-PAGE

Sample: Active recombinant MCP1, Human

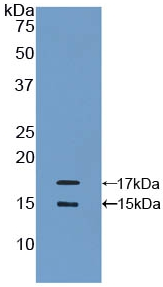

Figure 5. Western Blot

Sample: Recombinant MCP1, Human;

Antibody: Rabbit Anti-Human MCP1 Ab (PAA087Hu01)

[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.