

APA087Mu61 2mg
Active Monocyte Chemotactic Protein 1 (MCP1)
Organism Species: *Mus musculus (Mouse)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Gln24~Asn148

Tags: N-terminal His-tag

Purity: >90%

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

Buffer Formulation: PBS, pH7.4, containing 5% trehalose.

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 9.8

Predicted Molecular Mass: 15.4kDa

Accurate Molecular Mass: 22-35kDa 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 (pH7.6) 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 PLTCCYSFTS KMIPMSRLES  
YKRITSSRCP KEAVVFVTKL KREVCADPKK EWVQTYIKNL DRNQMRSEPT  
TLFKTASALR SSAPLNVKLT RKSEANASTT FSTTTSSSTSV GVTSVTVN
```

[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. Mouse 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 mouse MCP1 on the THP-1 cell line. Briefly, THP-1 cells were seeded into the upper chambers (150 ul cell suspension, 10^6 cells/ml in RPMI 1640 with FBS free) and different concentrations of recombinant mouse MCP1 diluted with serum free RPMI 1640 was added in lower chamber with a polycarbonate filter (8 um pore size) used to separate the two compartments.

After incubation at 37 °C with 5% CO₂ 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 recombinant mouse MCP1 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. Statistical results were shown in Figure 2. The optimum chemotaxis of MCP1 occurs at 0.1 ng/ml.

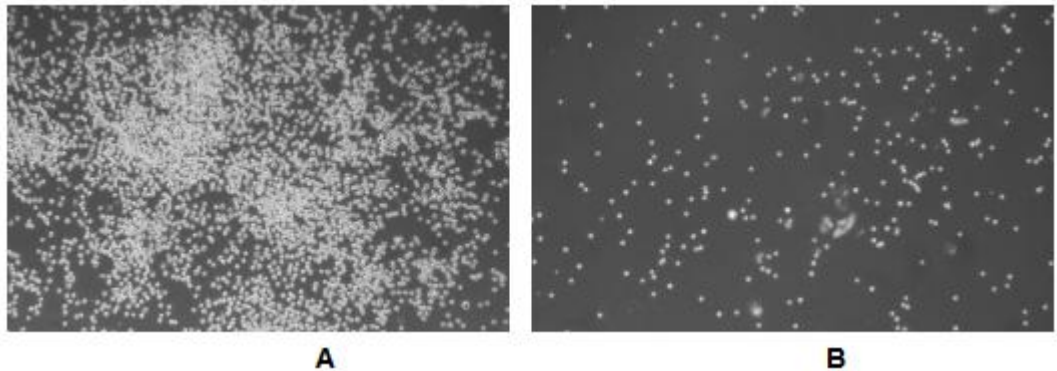


Figure 1. The chemotactic effect of recombinant mouse MCP1 on THP-1 cells

- (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 (×100) after incubation for 1h;
- (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 (×100) after incubation for 1h.

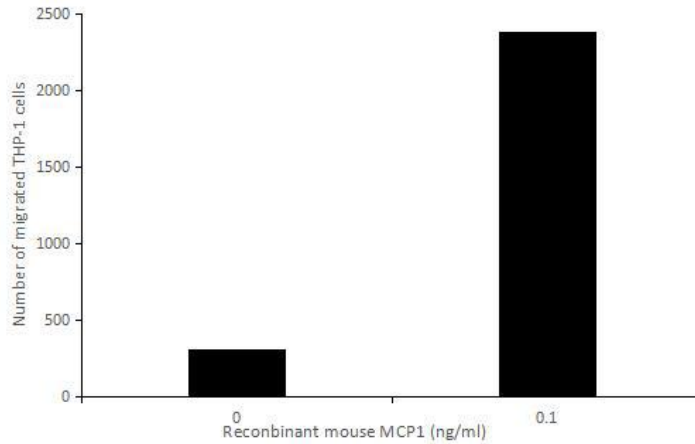


Figure 2. The chemotactic effect of recombinant mouse MCP1 on THP-1 cells

[IDENTIFICATION]

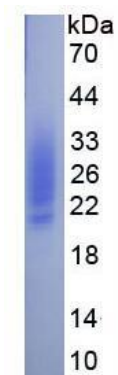


Figure 3. SDS-PAGE

Sample: Active recombinant MCP1, Mouse

[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.