APA092Hu61 100μg
Active Macrophage Inflammatory Protein 1 Alpha (MIP1a)
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: 293F cell

Residues: Ser24~Ala92

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: 4.8

Predicted Molecular Mass: 9.3kDa

Accurate Molecular Mass: 14kDa 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]

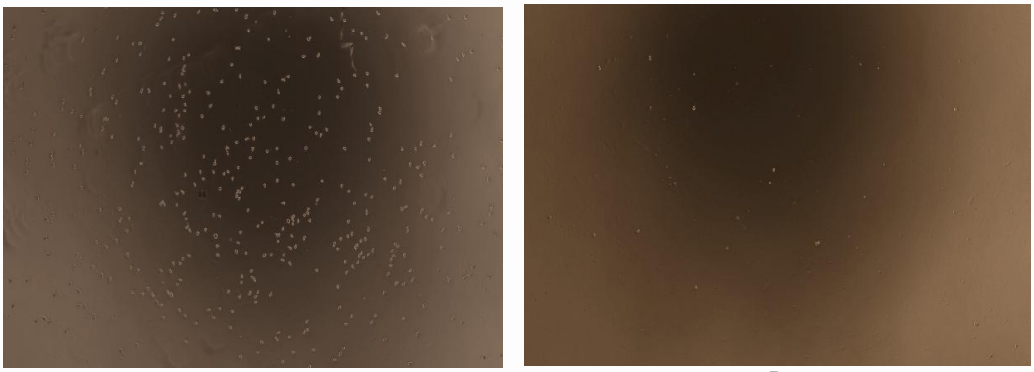
SLAADTP TACCFSYTSR QIPQNFIADY FETSSQCSKP GVIFLTKRSR QVCADPSEEW VQKYVSDLEL SA

[ACTIVITY]

MIP-1a (macrophage inflammatory protein 1-alpha) also known as Chemokine (C-C motief) ligand 3 (CCL3), is a cytokine belonging to the CC chemokine family that is involved in the recruitment and activation of macrophages, monocytes and neutrophils. In this case, chemotaxis assay used 24-well microchemotaxis system was undertaken to evaluate the chemotactic effect of MIP-1a on the human monocytic cell line THP1. Briefly, THP1 cells were seeded into the upper chambers (100μl cell suspension, 10^6 cells/ml in RPMI 1640 with 0.5% FBS) and MIP-1a (100ng/mL, diluted 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% CO_2 for 5h, the filter was removed, then cells in low chamber were observed by inverted microscope at low magnification (×40) and the number of migrated cells were counted at high

magnification (×400) randomly (five fields for each filter).

Result 1: By counting migrated cells in low chamber at high magnification (×400) randomly, it was shown that a mean of 41.2 THP1 cells/field migrated towards serum free RPMI 1640 medium with 100ng/mL MIP-1a, while only 3.6 THP1 cells/field migrated towards serum free RPMI 1640 medium. And the migrated THP1 cells in low chamber at low magnification (×40) was shown in Figure 1.



A B

Figure 1. The chemotactic effect of MIP-1-alpha on THP1 cells

(A) THP1 cells were seeded into the upper chambers and serum free RPMI 1640 with 100ng/mL MIP-1a was added in lower chamber, then cells in lower chamber were observed at low magnification (×40) after incubation for 5h;

(B) THP1 cells were seeded into the upper chambers and serum free RPMI 1640 with no MIP-1a was added in lower chamber, then cells in lower chamber were observed at low magnification (×40) after incubation for 5h.

[IDENTIFICATION]

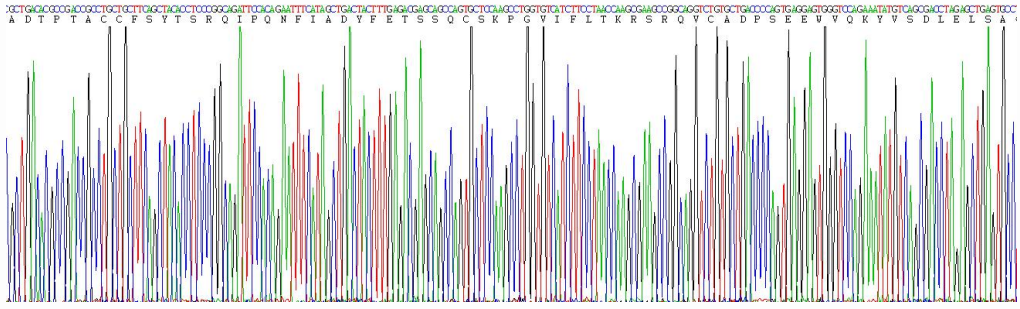


Figure 2. Gene Sequencing (extract)

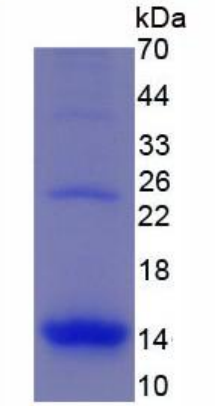


Figure 3. SDS-PAGE

Sample: Active recombinant MIP1a, Human

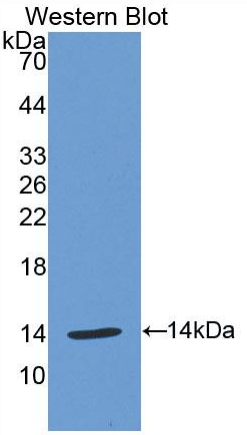


Figure 4. Western Blot

Sample: Recombinant MIP1a, Human;

Antibody: Rabbit Anti-Human MIP1a Ab (PAA092Hu06)