APA095Hu01 50μg
Active Macrophage Inflammatory Protein 3 Alpha (MIP3a)
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: Ala27~Met96
Tags: N-terminal His-tag
Purity: >95%
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: 9.6
Predicted Molecular Mass: 9.3kDa
Accurate Molecular Mass: 13kDa 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 (pH7.4) 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]

ASNF DCCLGYTDRI LHPKFIVGFT
RQLANEGCDI NAIIFHTKKK LSVCANPKQT WVKYIVRLLS KKVKNM

[ACTIVITY]

Macrophage Inflammatory Protein 3 Alpha (MIP-3α), also known as LARC (liver and activation-regulated chemokine), Exodus-1 or CCL20, is a CC chemokine with a selective chemotactic activity for lymphocytes and dendritic cells (DCs). MIP3α is produced by activated cells, including monocytes, T cells, endothelial cells, epithelial cells, and fibroblasts and is expressed in liver, lung, and some lymphoid tissues. This chemokine elicits its effects on its target cells by binding to the chemokine receptor CCR7. It attracts certain cells of the immune system, including dendritic cells and antigen-engaged B cells, CCR7+ central-memory T-Cells. Thus, chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the chemotactic effect of recombinant human MIP-3α on the Jurkat cell line. Briefly, Jurkat cells were seeded into the upper chambers

(200μL cell suspension,10^6 cells/mL in RPMI 1640 with FBS free) and MIP-3α (0.01ng/mL, 0.1ng/mL, 1ng/mL, 10ng/mL,100ng/mL and 1000ng/mL diluted separately in serum free RPMI 1640) was added in lower chamber with a polycarbonate filter (8 μm pore size) used to separate the two compartments. After incubation at 37℃ with 5% CO_2 for 2h, 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 MIP-3α is able to induce migration of Jurkat cells. The migrated Jurkat 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 results were shown in Figure 2. The optimum chemotaxis of recombinant human MIP-3α occurs at 1-10ng/mL.

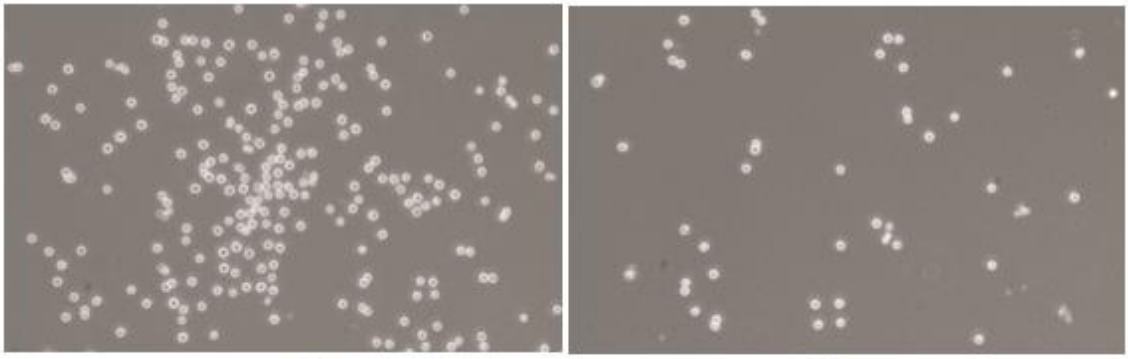

Figure 1. The chemotactic effect of recombinant human MIP-3 α on Jurkat cells

(A) Jurkat cells were seeded into the upper chambers and serum free RPMI 1640 with 10ng/mL MIP-3α was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 2h;

(B) Jurkat cells were seeded into the upper chambers and serum free RPMI 1640 without MIP-3α was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 2h.

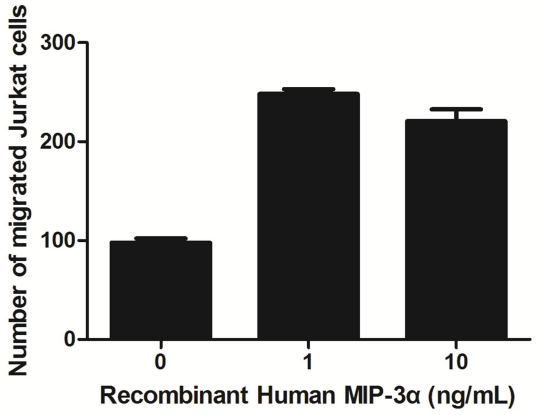

Figure 2. The chemotactic effect of recombinant human MIP-3α on Jurkat cells

[IDENTIFICATION]

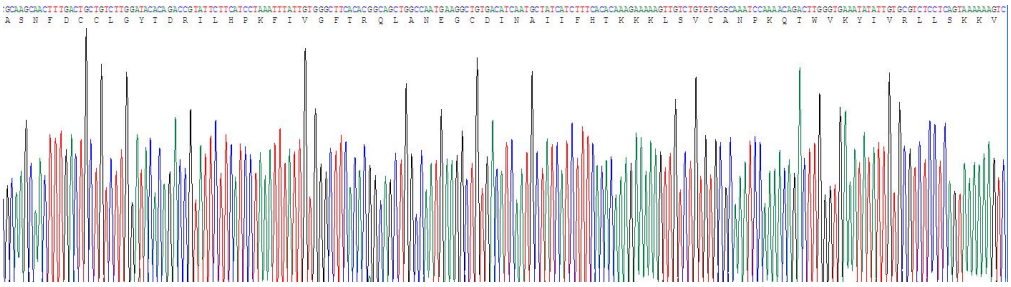

Figure 3. Gene Sequencing (extract)

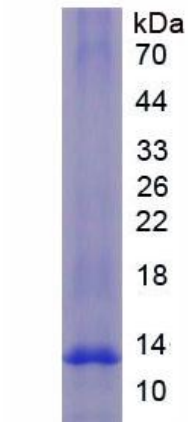

Figure 4. SDS-PAGE

Sample: Active recombinant MIP3a, Human

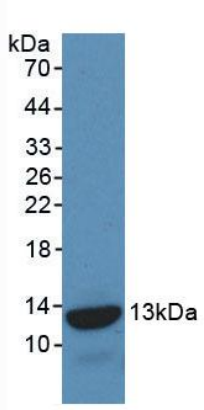

Figure 5. Western Blot

Sample: Recombinant MIP3a, Human;

Antibody: Rabbit Anti-Human MIP3a Ab (PAA095Hu01)

[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.