APA096Hu01 100μg
Active Active Macrophage Inflammatory Protein 3 Beta (MIP3b)
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: Gly22~Ser98
Tags: N-terminal His-tag
Purity: >98%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 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: 10.1kDa
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.

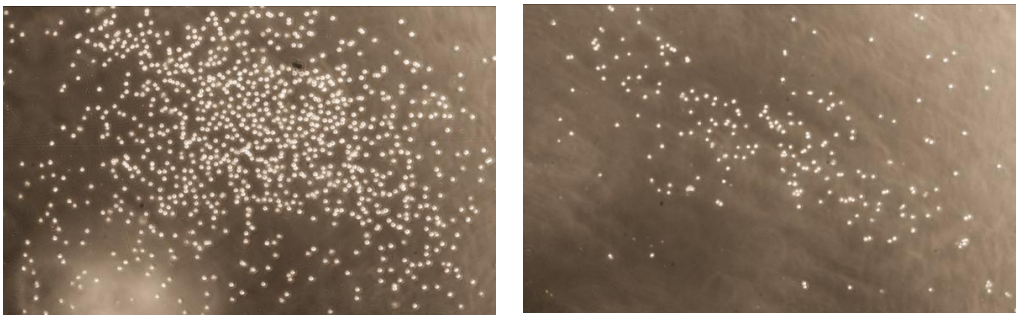
[SEQUENCE]

GTNDAEDCC LSVTQKPIPG YIVRNFHYLL IKDGCRVPAV VFTTLRGRQL CAPPDQPWVE RIIQRLQRTS AKMKRRSS

[ACTIVITY]

Macrophage Inflammatory Protein 3 Beta (MIP3b) is a small cytokine belonging to the CC chemokine family that is also known as EBI1 ligand chemokine (ELC) and Chemokine C-C motif ligand 19 (CCL19). This chemokine elicits its effects on its target cells by binding to the chemokine receptor 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 MIP3b on the Jurkat cell line. Briefly, Jurkat cells were seeded into the upper chambers (150μL cell suspension,10^6 cells/mL in RPMI 1640 with FBS free) and MIP3b (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 (8μm pore size) used to separate the two compartments. After incubation at 37℃ 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 shows MIP3b 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 MIP3b occurs at 0.1-1ng/mL.



A B

Figure 1. The chemotactic effect of recombinant human MIP3b on Jurkat cells.

(A) Jurkat cells were seeded into the upper chambers and serum free RPMI 1640 with 0.1ng/mL MIP3b was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 3h;

(B) Jurkat cells were seeded into the upper chambers and serum free RPMI 1640 without MIP3b was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 3h.

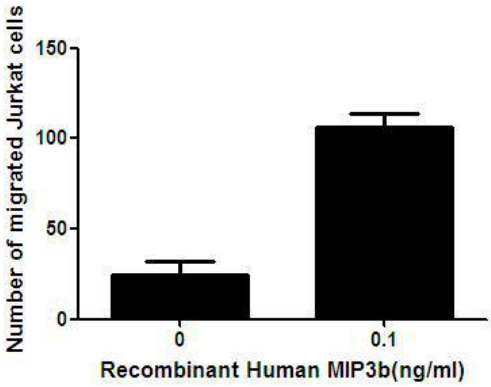


Figure 2. The chemotactic effect of recombinant human MIP3b on Jurkat cells.

[IDENTIFICATION]

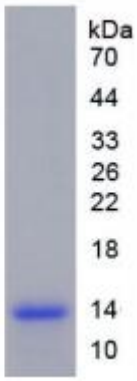


Figure 3. SDS-PAGE

Sample: Active recombinant MIP3b, Human

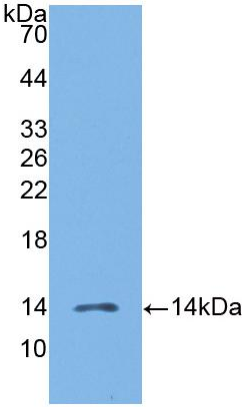


Figure 4. Western Blot

Sample: Recombinant MIP3b, Human;

Antibody: Rabbit Anti-Human MIP3b Ab (PAA096Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.