APA698Mu01 100µg Active Macrophage Migration Inhibitory Factor (MIF) Organism Species: *Mus musculus (Mouse) 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: Phe4~Ser112 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 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: 6.7

Predicted Molecular Mass: 13.1kDa

Accurate Molecular Mass: 14kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

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.

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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]

FIVNTNV PRASVPEGFL SELTQQLAQA TGKPAQYIAV HVVPDQLMTF SGTNDPCALC SLHSIGKIGG AQNRNYSKLL CGLLSDRLHI SPDRVYINYY DMNAANVGWN GS

[ACTIVITY]

Macrophage migration inhibitory factor (MIF), also known as glycosylation-inhibiting factor (GIF), L-dopachrome isomerase, or phenylpyruvate tautomerase is a protein classified as an inflammatory cytokine. MIF is an important regulator of innate immunity. It involved in cell-mediated immunity, immunoregulation, and inflammation. MIF plays a role in the regulation of macrophage function in host defense through the suppression of anti-inflammatory effects of glucocorticoids. This lymphokine and the JAB1 protein form a complex in the cytosol near the peripheral plasma membrane, which may indicate a role in integrin signaling pathways. Besides, Major Histocompatibility Complex Class II Invariant Chain (MHCDG) has been identified as an interactor of MIF, thus a binding ELISA assay was conducted to detect the interaction of recombinant mouse MIF and recombinant mouse MHCDG. Briefly, MIF were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to MHCDG-coated microtiter wells and incubated for 2h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-MIF pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells

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were incubated 15-25 minutes at $37 \,^{\circ}$ C. Finally, add 50μ L stop solution to the wells and read at 450nm immediately. The binding activity of MIF and MHCDG was shown in Figure 1, and this effect was in a dose dependent manner.



Figure 1. The binding activity of MIF with MHCDG.

[IDENTIFICATION]



Figure 2. SDS-PAGE Sample: Active recombinant MIF, Mouse

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Figure 3. Western Blot Sample: Recombinant MIF, Mouse; Antibody: Rabbit Anti-Mouse MIF Ab (PAA698Mu01)

[<u>IMPORTANT NOTE</u>]

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.