

Human MIF protein, His tag (active)

Cat. No. GTX00196-pro

Application	Functional Assay
Species	Human

Package
10 µg

APPLICATION

Application Note

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 human MIF and recombinant human MHCDG. Briefly, MIF were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl 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 were incubated 15-25 minutes at 37°C. Finally, add 50 µl stop solution to the wells and read at 450nm immediately. The binding activity of MIF and MHCDG was in a dose dependent manner.

Macrophage migration inhibitory factor (MIF or MMIF), is a lymphokine involved in cell-mediated immunity, immunoregulation, and inflammation. MIF contains two motifs with catalytic activity. The first is a 27 amino acid motif located at the N-terminus functions as a phenylpyruvate tautomerase. MIF also contains a Cys-Ala-Leu-Cys catalytic site between residues 57 and 60 that appears to function as a disulfide reductase. Besides, MIF is overexpressed in various tumors and has been suggested as a molecular link between chronic inflammation and cancer. To measure the effect of MIF on cell proliferation, breast cancer MCF-7 cells were seeded into triplicate wells of 96-well plates at a density of 5000 cells/well and allowed to attach, replaced with serum-free overnight, then the medium was replaced with 1% serum standard DMEM prior to the addition of various concentrations of recombinant human MIF. After incubated for 96h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 µl of CCK-8 solution was added to each well of the plate, then the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37°C. Proliferation of MCF-7 cells after incubation with MIF for 96h observed by inverted microscope. Cell viability was assessed by CCK-8 assay after incubation with recombinant MIF for 96h. And MIF significantly increased cell viability of MCF-7 cells.

Observed MW 14 kDa.

PROPERTIES

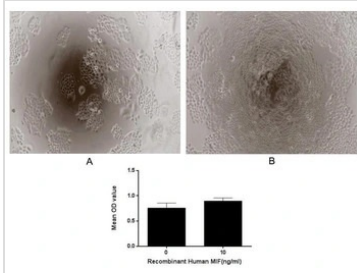
Form	Lyophilized powder
Buffer	Reconstitute with 20mM Tris and 150mM NaCl to 0.1-1.0mg/ml. Do not vortex. Lyophilized from 20mM Tris, 150mM NaCl, 1mM EDTA, 1mM DTT, 0.01% SKL, 5% Trehalose.
Preservative	ProClin 300
Storage	For short-term storage (1-2 weeks), store at 4°C. For long-term storage, store at -20°C or below. After reconstitution, keep as concentrated solution. Avoid freeze-thaw cycles.
Region/Sequence	N-terminal His-Tag; Met3~Asn111 (NP_002406.1)
Expression System	E. coli
Purity	> 90%



For full product information, images and publications, please visit our [website](#).

Date 2024 / 05 / 06 Page 1 of 2

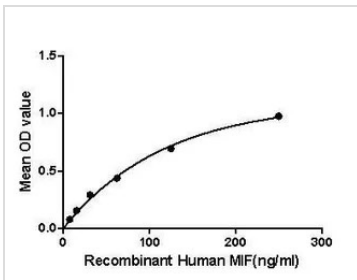
Endotoxin	< 1 EU/μg
Conjugation	Unconjugated
Note	For laboratory use only. Not for any clinical, therapeutic, or diagnostic use in humans or animals. Not for animal or human consumption.

DATA IMAGES

GTX00196-pro Functional Assay Image

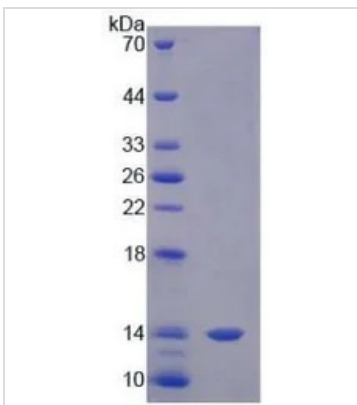
Cell proliferation effect of GTX00196-pro Human MIF protein (active). Cell viability was measured by Cell Counting Kit-8 (CCK-8).

(A) Unstimulated MCF-7 cells cultured in DMEM for 96hrs.

(B) MCF-7 cells cultured in DMEM, stimulated with 10 ng/ml MIF for 96hrs.


GTX00196-pro Functional Assay Image

Functional ELISA analysis of GTX00196-pro Human MIF protein (active) which can bind immobilized MHCDG protein.


GTX00196-pro Image

SDS-PAGE analysis of GTX00196-pro Human MIF protein (active).



For full product information, images and publications, please visit our [website](https://www.genetex.com).