

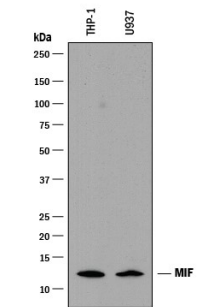
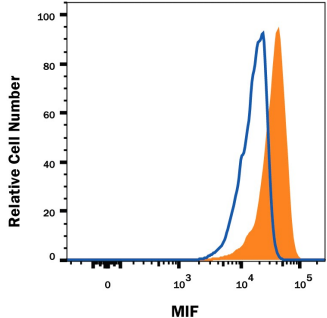
DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human MIF in direct ELISAs.
Source	Monoclonal Mouse IgG ₁ Clone # 932606
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human MIF Met1-Phe114 Accession # P14174
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Intracellular Staining by Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
CytoF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Western Blot	THP-1 human acute monocytic leukemia cell line and U937 human histiocytic lymphoma cell line with 2 µg/mL of Mouse Anti-Human MIF Monoclonal Antibody (Catalog # MAB2891). It is recommended to use Mouse Anti-Human MIF Monoclonal Antibody (Catalog # MAB2893, Clone # 932603) as an alternative antibody for Western blot.	

DATA

<p>Western Blot</p> 	<p>Detection of Human MIF by Western Blot. Western blot shows lysates of THP-1 human acute monocytic leukemia cell line and U937 human histiocytic lymphoma cell line. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human MIF Monoclonal Antibody (Catalog # MAB2891) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for MIF at approximately 12 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1. It is recommended to use Mouse Anti-Human MIF Monoclonal Antibody (Catalog # MAB2893, Clone # 932603) as an alternative antibody for Western blot.</p>	<p>Intracellular Staining by Flow Cytometry</p> 	<p>Detection of MIF in Human PBMCs by Flow Cytometry. Human peripheral blood mononuclear cell (PBMCs), resting (open histogram), or treated with 1 µg/mL LPS overnight and 3 µM monensin for 2 hours (filled histogram) were stained with Mouse Anti-Human MIF Monoclonal Antibody (Catalog # MAB2891) or isotype control antibody (Catalog # MAB002), followed by Phycoerythrin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0102B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer 1 (Catalog # FC005).</p>
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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

MIF (or macrophage Migration Inhibitory Factor) was the first lymphokine/cytokine to be recognized in the pregenomics era (1, 2). Regardless, it is one of the least understood of all inflammatory mediators (1, 3). Human MIF is a 12.5 kDa, 115 amino acid (aa) nonglycosylated polypeptide that is synthesized without a signal sequence (4-7). Secretion occurs nonclassically via an ABCA1 transporter (8). The initiating Met is removed, leaving Pro as the first amino acid. The molecule consists of two α -helices and six β -strands, four of which form a β -sheet. The two remaining β -strands interact with other MIF molecules, creating a trimer (2, 9, 10). Structure-function studies suggest MIF is bifunctional with segregated topology. The N- and C-termini mediate enzyme activity (in theory). Phenylpyruvate tautomerase activity (enol-to-keto) has been demonstrated and is dependent upon Pro at position #1 (11). Amino acids 50-65 have also been suggested to contain thiol-protein oxidoreductase activity (12). MIF has proinflammatory cytokine activity centered around aa's 49-65. On fibroblasts, MIF induces, IL-1, IL-8, and MMP expression; on macrophages, MIF stimulates NO production and TNF- α release following IFN- γ activation (13, 14). MIF apparently acts through CD74 and CD44, likely in some form of trimeric interaction (15, 16). Human MIF is active on mouse cells (14). Human MIF is 90%, 94%, 95%, and 90% aa identical to mouse, bovine, porcine, and rat MIF, respectively.

References:

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