

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat MIF in Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human MIF Pro2-Ala115 Accession # AAA36315
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.

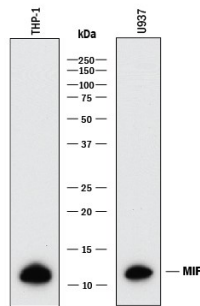
APPLICATIONS

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
Western Blot	0.5 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10 ⁶ cells	HL-60 human acute promyelocytic leukemia cell line treated with Camptothecin, fixed with paraformaldehyde, and permeabilized with saponin
Simple Western	10 µg/mL	THP-1 human acute monocytic leukemia cell line
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

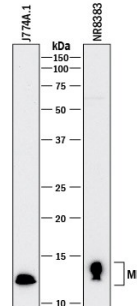
DATA

Western Blot



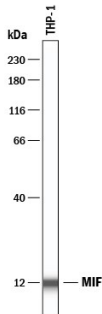
Detection of Human MIF by Western Blot. Western blot shows lysates of THP-1 human acute monocytic leukemia cell line and U937 human histiocytic lymphoma cell line. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human/Mouse/Rat MIF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-289-PB) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). 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](#).

Western Blot



Detection of Mouse and Rat MIF by Western Blot. Western blot shows lysates of J774A.1 mouse reticulum cell sarcoma macrophage cell line and NR8383 rat alveolar macrophage cell line. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human/Mouse/Rat MIF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-289-PB) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for MIF at approximately 12-14 kDa (as indicated). This experiment was conducted under reducing conditions and using [Immunoblot Buffer Group 1](#).

Simple Western



Detection of Human MIF by Simple Western™. Simple Western lane view shows lysates of THP-1 human acute monocytic leukemia cell line, loaded at 0.2 mg/mL. A specific band was detected for MIF at approximately 12 kDa (as indicated) using 10 µg/mL of Goat Anti-Human/Mouse/Rat MIF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-289-PB). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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 non-classically 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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