

Human macrophage migration inhibitory factor (MIF) ELISA Kit Datasheet: OI0005

Application:

Human MIF ELISA Kit is designed to measure MIF in various samples including serum, plasma and the supernatant from different cell cultures. The assay will recognize both natural and recombinant Human MIF.

Test principle:

MIF is a protein of 115 amino acids that is expressed in a wide variety of tissues. The nature forms of MIF are a mixture of monomeric, dimeric, and trimeric forms, whereas only monomeric MIF was detected when MIF was electrophoresed without prior cross-linking (1). The biologically active forms of MIF are dimer and trimer, which contain identical subunits linked together by the hydrogen bonds (2-4). Our cross-linking study with MIF from different resources demonstrated that the average ratio of monomer, dimer and trimer is 74:20:6 (Figure 1).

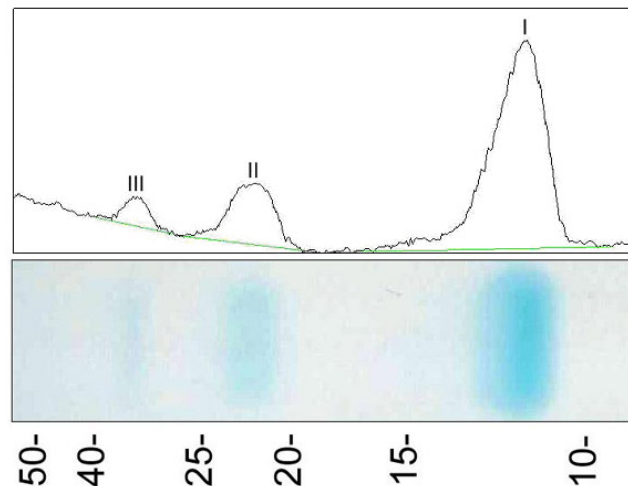


Fig. 1. The human MIF protein was crosslinked with glutaraldehyde. After SDS-PAGE, the gel was stained with GelCode Blue (PI24590, Thermo Scientific) and the image was analyzed with ImagJ1.43i (Wayne Rasband, NIH).

The human MIF ELISA kit detects both dimer and trimer forms of MIF in the samples (Fig. 2). Therefore, the total amounts of MIF as labeled on the standard samples are quantified based on the ratios of multimer in the total MIF, which is about 26%. The standard reference is used to determine the MIF concentration of the unknowns.

Materials Provided:

The kit contains sufficient materials to yield approximately five 96-well plates if the recommended storage, materials, buffer and protocol are followed as specified in this package:

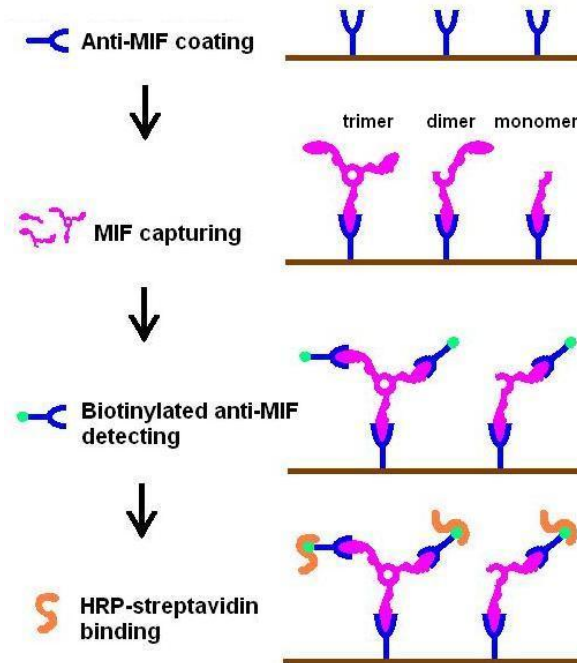


Fig.2 Schema of the assay procedure.

Capture antibody

Anti-human MIF monoclonal antibody ([OI0004](#)). Store at -20°C.

Detection antibody

Biotinylated anti-human MIF monoclonal antibody, 50µl. Store at 2-8°C.

Enzyme Reagent

Streptavidin-horseradish peroxidase conjugate (SAv-HRP), 50µl. Store at 2-8°C.

Standard

Human MIF (100ng/ml), 250µl, Store at -20°C.

Expiration: 6 months after the date of shipment.

Materials Required:

Coating buffer: 0.1M Sodium Carbonate, pH9.5 (8.40g NaHCO₃, 3.56g Na₂CO₃; q.s. to 1.0L and adjust to pH 9.5).

Washing buffer: PBST (1XPBS, 0.05% Tween 20).

Substrate solution: Tetramethylbebidine (TMB) is recommended.

Stop solution: 2M hydrochloric acid.

96-well plate: BD Falcon #353279 or equivalent is recommended.

Recommended Assay Procedures:

1. Coat 96-well plate with 100µl per well of capture antibody diluted in coating buffer (2µg/ml). Seal plate and incubate overnight at 4°C.
2. Aspirate wells and do the "1+3" washes, i.e. add washing buffer 250ul/well, aspirate immediately, 1 brief wash; then add washing buffer and incubate at room temperature (RT) for 3 min, aspirate and repeat two times, 3 incubation washes. For each wash, invert plate and blot on absorbent paper to remove residual buffer.
3. Fill the well with PBST, incubate at RT for 30 min. *Note: During the incubation, prepare the standard (recommend starting from 10ng/ml) and sample dilution in PBST.*

4. Aspirate the wells.
5. Add the prepared standard and samples 100 μ l/well into appropriate wells in the duplicate or triplicate manner. Seal plate and incubate at RT for 1-2 hours.
6. Aspirate and do the "1+3" washes.
7. Add the detection antibody diluted in PBST (1:1000 dilution). Seal plate and incubate at RT for 1-2 hours.
8. Aspirate and do the "1+3" washes.
9. Add 100 μ l/well of the enzyme reagent (SAV-HRP) diluted with PBST (1:1000-1:2000 dilution) into each well, seal plate and incubate at RT for 1 hour.
10. Aspirate and do a "1+5" washes as above by 5 incubation washes instead of 3.
11. Add 100 μ l of substrate solution to each well. Incubate plate at RT for 15 min in the dark.
12. Termination with 50 μ l/well 2M hydrochloric acid and read absorbance at 450nm within 20 minutes.

Calculation of Results:

1. Calculate the mean luminescence for each set of standard wells.
2. A standard curve is generated by plotting the mean luminescence (y axis) against ng/ml standard (x axis).
3. Read the MIF concentration for unknown samples from the standard curve plotted in step 2. For best results, a 4-parameter logistic curve-fit (sigmoidal dose-response with variable slope) is recommended.

Typical Standard Curve:

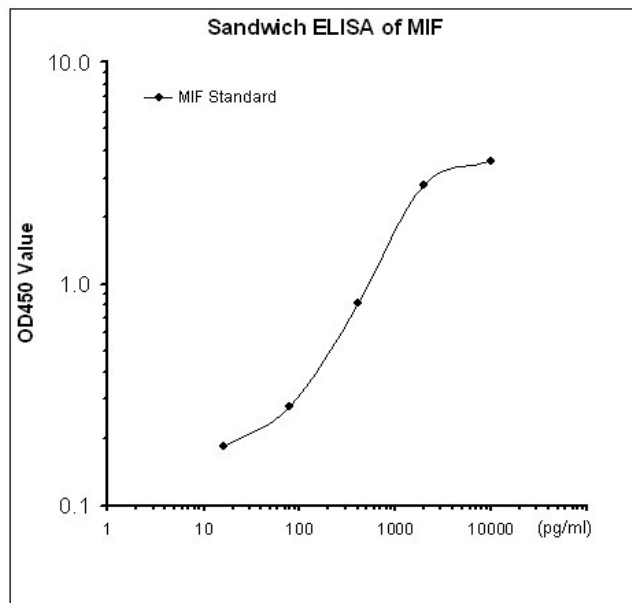


Fig. 3. The example of a typical standard curve. This standard curve is for demonstration only. A standard curve must be run with each assay.

References:

1. Sun, H.W., J. Bernhagen, R. Bucala, and E. Lolis. 1996. Crystal structure at 2.6-A resolution of human macrophage migration inhibitory factor. *Proceedings of the National Academy of Sciences of the United States of America* 93:5191-5196.
2. Sugimoto, H., M. Suzuki, A. Nakagawa, I. Tanaka, and J. Nishihira. 1996. Crystal structure of macrophage migration inhibitory factor from human lymphocyte at 2.1 A resolution. *FEBS letters* 389:145-148.
3. Suzuki, M., H. Sugimoto, A. Nakagawa, I. Tanaka, J. Nishihira, and M. Sakai. 1996. Crystal structure of the macrophage migration inhibitory factor from rat liver. *Nature structural biology* 3:259-266.
4. Mischke, R., R. Kleemann, H. Brunner, and J. Bernhagen. 1998. Cross-linking and mutational analysis of the oligomerization state of the cytokine macrophage migration inhibitory factor (MIF). *FEBS letters* 427:85-90.
5. Zhou H, Wang Y, Wang W, Jia J, Li Y, Wang Q, Wu Y, Tang J. Generation of monoclonal antibodies against highly conserved antigens. *PLoS One*. 2009 Jun 30; 4(6): e6087.

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Address:

OncoImmune Inc.
333 Parkland Plaza Suite 1000
Ann Arbor, MI 48103
Tel: 734-332-4234
Fax: 734-864-5777
Email: xzheng@oncoimmune.com
Web: www.oncoimmune.com