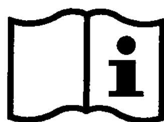
Product information

IBL AMERICA



User´s Manual

Human MIF ELISA

For the precise measurement of MIF in human serum, plasma, body fluids, tissue homogenate or cell culture supernates.

BE69185

96

Storage: 2-8°C

RUO **For Research Use Only – Not for Use in Diagnostic Procedures.**

Manufactured for:
Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave NE, Suite P, Minneapolis, MN 55432
Toll Free: (888) 523-1246 Fax: (763) 780-2988
www.ibl-america.com / info@ibl-america.com

1 INTRODUCTION

1.1 Intended Use

The IBL-America MIF ELISA has been designed for the precise measurement of MIF in human serum, plasma, body fluids, tissue homogenate or cell culture supernates. For research use only, not for use in diagnostic procedures.

1.2 Background

Macrophage migration inhibitory factor (MIF or MMIF) also known as glycosylation-inhibiting factor (GIF), L-dopachrome isomerase, or phenylpyruvate tautomerase is a protein that in humans is encoded by the MIF gene. Macrophage migration inhibitory factor assembles into a trimer composed of three identical subunits. Each of these monomers contain two antiparallel alpha helices and a four-stranded beta sheet. The monomers surround a central channel with 3-fold rotational symmetry. MIF plays a role in the regulation of macrophage function in host defense through the suppression of anti-inflammatory effects of glucocorticoids. It is an inflammatory mediator associated with rheumatoid arthritis (RA) severity. Additionally, evidence suggests that there is a correlation between MIF production and metastatic potential in colorectal cancer.

2 PRINCIPLE OF THE TEST

This kit is based on a sandwich enzyme-linked immune-sorbent assay technology. An analyte-specific polyclonal antibody is pre-coated onto 96-well plates. The biotin conjugated second antibody is used as detection antibody. During the first incubation the standards and samples react with the analyte-specific pre-coated antibody. In a second incubation the biotin conjugated detection antibody completes the sandwich. After washing with wash buffer the TMB substrate is added to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The optical density of the yellow color is proportional to the analyte captured in plate. Read the O.D. absorbance at 450nm in a microtiterplate reader, and calculate the concentration of the analyte in the sample by taking into consideration the dilution factor of the sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only, not for use in diagnostic procedures.
2. Before the experiment, centrifuge each kit component for several minutes to bring down all reagents to the bottom of tubes.
3. It is recommend to measure each standard and sample in duplicate.
4. Do NOT let the plate completely dry at any time! Since the dry condition can inactivate the biological material on the plate.
5. Do not reuse pipette tips and tubes to avoid cross contamination.
6. Do not use the expired components or the components from different lot numbers.
7. To avoid the marginal effect of plate incubation for temperature differences (the marginal wells always get stronger reaction), it is recommend to equilibrate the ABC working solution and TMB substrate for at least 30 min at 37°C before adding to wells.
8. The TMB substrate (Kit Component 8) is colorless and transparent before use. If not, please contact us for replacement.

4 REAGENTS

4.1 Reagents provided

1. One 96-well microtiterplate pre-coated with anti-human MIF antibody
2. Lyophilized human MIF standards: 2 tubes (10 ng / tube)
3. Sample / Standard diluent buffer: 30 ml
4. Biotin conjugated anti-human MIF antibody (Concentrated): 130 µl. Dilution: 1:100
5. Antibody diluent buffer: 12 ml
6. Avidin-Biotin-Peroxidase Complex (ABC) (Concentrated): 130 µl. Dilution: 1:100
7. ABC diluent buffer: 12 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 30 ml

4.2 Materials required but not provided

1. 37°C incubator
2. Microtiterplate reader (wavelength: 450nm)
3. Precise pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml Eppendorf tubes
7. Plate cover
8. Absorbent filter papers
9. Plastic or glass container with volume of above 1 L

4.3 Storage Conditions / Expiration

Store at 4°C for frequent use, at -20°C for 8 months.

4.4 Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

✧ **Body fluids, tissue homogenate and cell culture supernatants:** Centrifuge to remove precipitate, analyze immediately or aliquot and store at -20°C.

✧ **Serum:** Coagulate the serum at room temperature (about 4 hours). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.

✧ **Plasma:** Collect plasma with heparin as the anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C. **Citrate and EDTA cannot be used as anticoagulant here.**

Note: 1. Coagulate blood samples completely, then, centrifuge, and avoid hemolysis and particles.
2. NaN_3 cannot be used as test sample preservative, since it is the inhibitor for HRP.

>> Sample Dilution Guideline

End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration falls the optimal detection range of the kit. Dilute the sample with the provided diluent buffer, and several trials may be necessary in practice. The test sample must be well mixed with the diluent buffer.

✧ **High target protein concentration (100-1000 ng/ml)**: Dilution: 1:100. i.e. Add 1 μl of sample into 99 μl of Sample / Standard diluent buffer (Kit Component 3).

✧ **Medium target protein concentration (10-100 ng/ml)**: Dilution: 1:10. i.e. Add 10 μl of sample into 90 μl of Sample / Standard diluent buffer (Kit Component 3).

✧ **Low target protein concentration (156-10,000 pg/ml)**: Dilution: 1:2. i.e. Add 50 μl of sample into 50 μl of Sample / Standard diluent buffer (Kit Component 3).

✧ **Very low target protein concentration (≤156 pg/ml)**: Unnecessary to dilute, or dilute at 1:2.

2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1:25) with distilled water (i.e. add 30ml of concentrated wash buffer into 720 ml of distilled water).

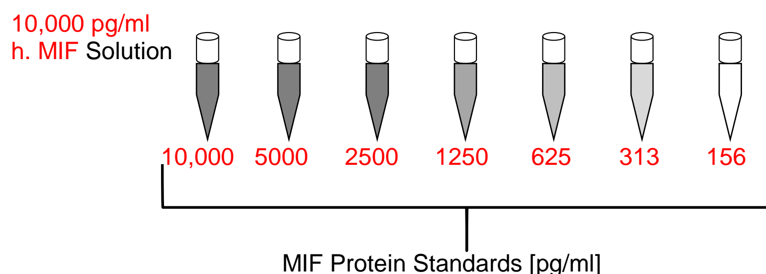
3. Standard

Reconstitution of the lyophilized human MIF standard (Kit Component 2): standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard are included in each kit. Use one tube for each experiment.
(Note: Do not dilute the standard directly in the plate)

a. 10,000 pg/ml of standard solution: Add **1 ml** of Sample / Standard diluent buffer (Kit Component 3) into one Standard (Kit Component 2) tube, keep the tube at room temperature for 10 min and mix thoroughly.

b. 5000 pg/ml → 156 pg/ml of standard solutions: Label 6 Eppendorf tubes with 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 313 pg/ml, 156 pg/ml, respectively. Aliquot **0.3 ml** of the Sample / Standard diluent buffer (Kit Component 3) into each tube. Add **0.3 ml** of the above 10,000 pg/ml standard solution into 1st tube and mix thoroughly. Transfer **0.3 ml** from 1st tube to 2nd tube and mix thoroughly. Transfer **0.3 ml** from 2nd tube to 3rd tube and mix thoroughly, and so on.

0.3ml 0.3 ml 0.3 ml 0.3 ml 0.3 ml 0.3 ml



Note: The standard solutions are best used within 2 hours. The 10,000 pg/ml standard solution should be used within 12 hours. Or store at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

4. Preparation of Biotin conjugated anti-human MIF antibody (Kit Component 4) working solution: prepare no more than 2 hours before the experiment.

a. Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

b. Dilute the Biotin conjugated anti-human MIF antibody (Kit Component 4) with Antibody diluent buffer (Kit Component 5) at 1:100 and mix thoroughly. i.e. Add 1 μl of Biotin conjugated anti-human MIF antibody into 99 μl of Antibody diluent buffer.

5. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) (Kit Component 6) working solution: prepare no more than 1 hour before the experiment.

a. Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1 - 0.2 ml more than the total volume)

b. Dilute the Avidin-Biotin-Peroxidase Complex (ABC) (Kit Component 6) with ABC diluent buffer (Kit Component 7) at 1:100 and mix thoroughly. i.e. Add 1 µl of Avidin-Biotin-Peroxidase Complex (ABC) into 99 µl of ABC diluent buffer.

5 ASSAY PROCEDURE

5.1 General Remarks

Before adding to wells, equilibrate the ABC working solution and TMB substrate (Kit Component 8) for at least 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

5.2 Test Procedure

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommend to measure each standard and sample in duplicate.
2. Aliquot 0.1 ml of 10,000 pg/ml, 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 313 pg/ml, 156 pg/ml standard solutions into the standard wells.
3. Add 0.1 ml of Sample / Standard diluent buffer (Kit Component 3) into the control (zero) well.
4. Add 0.1 ml of properly diluted sample (human serum, plasma, body fluids, tissue homogenate or cell culture supernatants) into test sample wells.
5. Seal the plate with a cover and incubate at 37°C for 90 min.
6. Remove the cover and discard the plate content, clap the plate on the absorbent filter papers or other absorbent material. **Do NOT let the wells completely dry at any time. Do not wash plate!**
7. Add 0.1 ml of Biotin conjugated anti-human MIF antibody work solution into the above wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37°C for 60 min.
9. Remove the cover, and wash plate 3 times with Wash buffer (Kit Component 10) using one of the following methods:

   Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer (Kit Component 10) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a **total of THREE washes**.

   Automated Washing: Aspirate all wells, then wash plate **THREE times** with Wash buffer (Kit Component 10) (overfilling wells with the buffer). After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min or shaking.
10. Add 0.1 ml of ABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
11. Remove the cover and wash plate 5 times with Wash buffer (Kit Component 10), and each time let the wash buffer stay in the wells for 1-2 min. (See Step 9 for plate wash method).
12. Add 0.1 ml of TMB substrate (Kit Component 8) into each well, cover the plate and incubate at 37°C in dark within 30 min. (**Note:** This incubation time is for reference use only, the optimal time should be determined by end user.) And the shades of blue can be seen in the first 3-4 wells (with most concentrated human MIF standard solutions), the other wells show no obvious color.
13. Add 0.1 ml of Stop solution (Kit Component 9) into each well and mix thoroughly. The color changes into yellow immediately.
14. Read the O.D. absorbance at 450 nm in a microtiterplate reader within 30 min after adding the stop solution.

5.3 Results

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human MIF concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

6 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use controls according to state and federal regulations. The use of controls is advised to assure the day to day validity of results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, results of unknowns should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IBL-America directly.

7 PERFORMANCE CHARACTERISTICS

7.1 Range

156 pg/ml -10,000 pg/ml

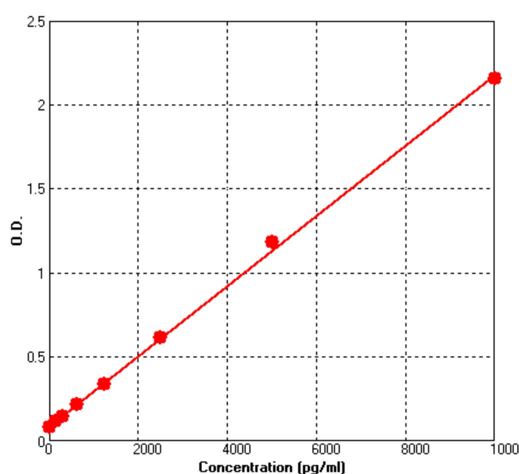
7.2 Sensitivity

<20 pg/ml

7.3 Typical Data & Standard Curve

Results of a typical standard run of a human MIF ELISA Kit are shown below**. This standard curve was generated at our lab for demonstration purpose only.** Each user should obtain their own standard curve as per experiment.
(N/A=not applicable)

X	pg/ml	0	156	313	625	1250	2500	5000	10,000
Y	OD450	0.084	0.117	0.145	0.218	0.337	0.613	1.181	2.154



7.4 References:

1.Weiser WY, Temple PA, Witek-Giannotti JS, Remold HG, Clark SC, David JR (October 1989). "Molecular cloning of a cDNA encoding a human macrophage migration inhibitory factor". Proc. Natl. Acad. Sci. U.S.A. 86 (19): 7522–6.
2.Sun HW, Bernhagen J, Bucala R, Lolis E (May 1996). "Crystal structure at 2.6-A resolution of human macrophage migration inhibitory factor". Proc. Natl. Acad. Sci. U.S.A. 93 (11): 5191–6.
3.Flaster H, Bernhagen J, Calandra T, Bucala R (June 2007). "The macrophage migration inhibitory factor-glucocorticoid dyad: regulation of inflammation and immunity". Mol. Endocrinol. 21 (6): 1267–80.
4.Bifulco C, McDaniel K, Leng L, Bucala R (2008). "Tumor growth-promoting properties of macrophage migration inhibitory factor". Curr. Pharm. Des. 14 (36): 3790–801.
5.He XX, Chen K, Yang J, Li XY, Gan HY, Liu CY, Coleman TR, Al-Abed Y (2009). "Macrophage migration inhibitory factor promotes colorectal cancer". Mol. Med. 15 (1-2): 1–10.

8 ORDERING INFORMATION

This kit is manufactured for Immuno-Biological Laboratories, Inc. (IBL-America). For ordering information, please contact:

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave NE, Suite P
Minneapolis, MN 55432
Toll Free: (888) 523-1246
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