

Human MPIF-1 ELISA Kit

User Manual

**Human MPIF-1
ELISA Kit Protocol**

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I. INTRODUCTION

MPIF-1 (Myeloid progenitor inhibitory factor-1) is also called Chemokine-beta-8, MIP-3-beta, or ELC. It is a highly basic protein ($pI > 9$) that is processed from a longer proprotein at the N-terminus. The protein exist as monomers under a variety of solution conditions. The factor is chemotactic for resting T-cells and monocytes. It is slightly chemotactic for neutrophils, and not chemotactic for activated T-lymphocytes. MPIF-1 is also a potent suppressor of bone marrow low proliferative potential colony-forming cells (LPP-CFC).

The Human MPIF-1 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human MPIF-1 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human MPIF-1 coated on a 96-well plate. Standards and samples are pipetted into the wells and MPIF-1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human MPIF-1 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of MPIF-1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

II. REAGENTS

1. MPIF-1 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human MPIF-1.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution
3. Standards (Item C): 2 vials, recombinant human MPIF-1.
4. Assay Diluent A (Item D): 30 ml of animal serum with 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.

5. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For Standard/Sample (cell culture medium/urine) diluent.
6. Detection Antibody MPIF-1 (Item F): 2 vial of biotinylated anti-human MPIF-1 (each vial is enough to assay half microplate).
7. HRP-Streptavidin Concentrate (Item G): 8 μ l of 10,000x concentrated HRP-conjugated streptavidin.
8. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.
9. Stop Solution (Item I): 8 ml of 2 M sulfuric acid.

III. STORAGE

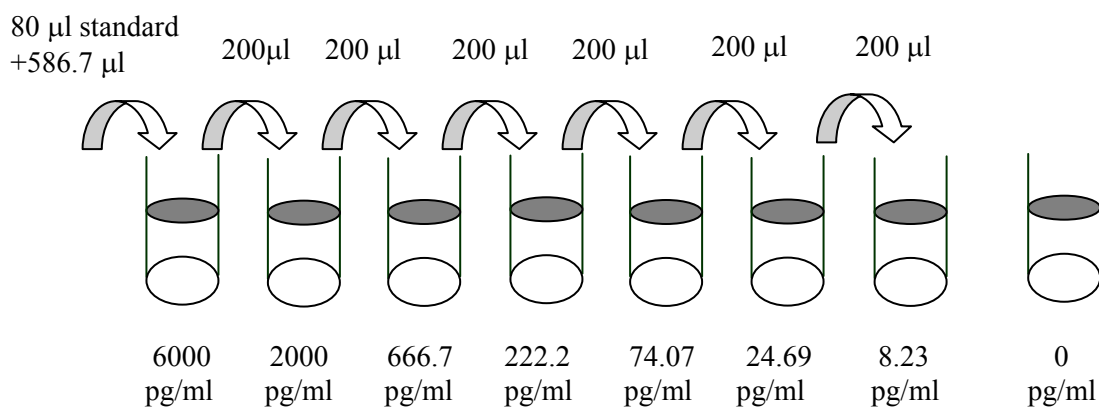
May be stored for up to 5 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20 °C or -80 °C (recommended at -80 °C) after reconstitution. Opened Microplate Wells and reagents may be store for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

IV. ADDITIONAL MATERIALS REQUIRED

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- 2 Precision pipettes to deliver 2 μ l to 1 ml volumes.
- 3 Adjustable 1-25 ml pipettes for reagent preparation.
- 4 100 ml and 1 liter graduated cylinders.
- 5 Absorbent paper.
- 6 Distilled or deionized water.
- 7 Log-log graph paper or computer and software for ELISA data analysis.
- 8 Tubes to prepare standard or sample dilutions.

V. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Preparation of standard: **Briefly spin the vial of Item C** and then add 400 μl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine, Assay Diluent B should be diluted 5-fold with deionized or distilled water) into Item C vial to prepare a 50 ng/ml standard. **Dissolve the powder thoroughly by a gentle mix.** Add 80 μl MPIF-1 standard from the vial of Item C, into a tube with 586.7 μl Assay Diluent A or 1x Assay Diluent B to prepare a 6,000 pg/ml stock standard solution. Pipette 400 μl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).



3. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) is used for dilution of serum/plasma samples, and Assay Diluent B (Item E) is used for dilution of culture supernatants and urine.

4. Assay Diluent B should be diluted 5-fold with deionized or distilled water.
5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 μ l of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) *and pipette up and down to mix gently* before use. HRP-Streptavidin concentrate should be diluted 10,000-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 2 μ l of HRP-Streptavidin concentrate into a tube with 198.0 μ l 1x Assay Diluent B to prepare a 100-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix through and then pipette 120 μ l of prepared 100-fold diluted solution into a tube with 12 ml 1x Assay Diluent B to prepare a final 10,000 fold diluted HRP-Streptavidin solution.

VI. ASSAY PROCEDURE:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 μl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C.
3. Discard the solution and wash 4 times with 1x Wash Solution (200 μl each).
4. Add 100 μl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature.
5. Discard the solution and wash 4 times with 1x Wash Solution (200 μl each).
6. Add 100 μl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature.
7. Discard the solution and wash 5 times with 1x Wash Solution (200 μl each).
8. Add 100 μl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark.
9. Add 50 μl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.



2. Add 100 μl standard or sample to each well.
Incubate 2.5 hours at room temperature or over night at 4°C.



3. Add 100 μ l prepared biotin antibody to each well.
Incubate 1 hour at room temperature.



4. Add 100 μ l prepared Streptavidin solution.
Incubate 45 minutes at room temperature.



5. Add 100 μ l TMB One-Step Substrate Reagent to each well.
Incubate 30 minutes at room temperature.



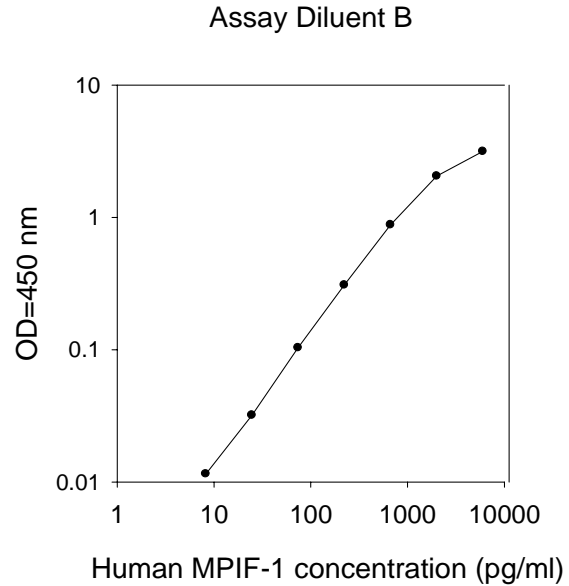
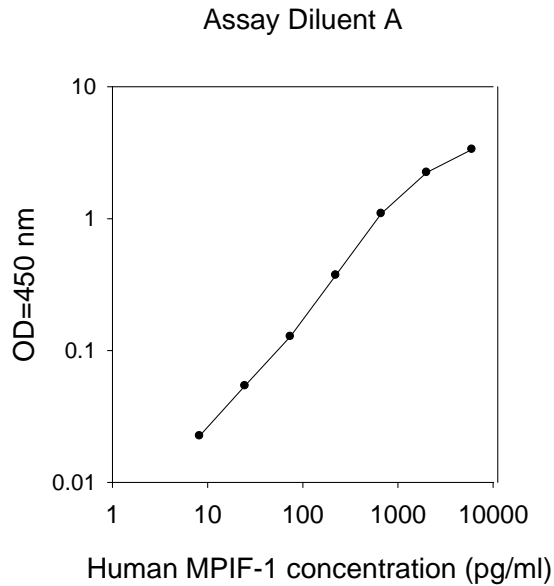
6. Add 50 μ l Stop Solution to each well.
Read at 450 nm immediately.

VIII. CALCULATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. TYPICAL DATA

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. SENSITIVITY

The minimum detectable dose of MPIF-1 is typically less than 7 pg/ml.

C. RECOVERY

Recovery was determined by spiking various levels of human MPIF-1 into human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	92.78	82-105
Plasma	94.16	83-106
Cell culture media	93.48	81-105

D. LINEARITY

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected Range (%)	88 80-103	90 81-104	92 82-103
1:4	Average % of Expected Range (%)	93 82-104	92 81-105	96 83-104
1:8	Average % of Expected Range (%)	91 81-102	94 83-105	95 82-103

E. REPRODUCIBILITY

Intra-Assay: CV<10%

Inter-Assay: CV<12%

IX. SPECIFICITY

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (*e.g.*, human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN- γ , Leptin, MCP-1, MCP-2, MCP-3, MDC, MIP-1 α , MIP-1 β , MIP-1 δ , PARC, PDGF, RANTES, SCF, TARC, TGF- β , TIMP-1, TIMP-2, TNF- α , TNF- β , TPO, VEGF).

X. REFERENCES:

1. Elagoz A et al A truncated form of CKbeta8-1 is a potent agonist for human formyl peptide-receptor-like 1 receptor. *British Journal of Pharmacology* 141(1): 37-46 (2004);
2. Patel PV et al Molecular and functional characterization of two novel human CC chemokines as inhibitors of two distinct classes of myeloid progenitors. *Journal of Experimental Medicine* 185(7): 1163-1172 (1997);
3. Youn BS et al Characterization of CKbeta8 and CKbeta8-1: two alternatively spliced forms of human beta-chemokine Forssmann U et al CKbeta8, a novel CC chemokine that predominantly acts on monocytes. *FEBS Letters* 408(2): 211-216 (1997);

XI. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
1. Poor standard curve	<ol style="list-style-type: none"> 1. Inaccurate pipetting 2. Improper standard dilution 	<ol style="list-style-type: none"> 1. Check pipettes 2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	<ol style="list-style-type: none"> 1. Too brief incubation times 2. Inadequate reagent volumes or improper dilution 	<ol style="list-style-type: none"> 1. Ensure sufficient incubation time; assay procedure step 2 may change to over night 2. Check pipettes and ensure correct preparation
3. Large CV	<ol style="list-style-type: none"> 1. Inaccurate pipetting 	<ol style="list-style-type: none"> 1. Check pipettes
4. High background	<ol style="list-style-type: none"> 1. Plate is insufficiently washed 2. Contaminated wash buffer 	<ol style="list-style-type: none"> 1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed. 2. Make fresh wash buffer
5. Low sensitivity	<ol style="list-style-type: none"> 1. Improper storage of the ELISA kit 2. Stop solution 	<ol style="list-style-type: none"> 1. Store your standard at $<-20^{\circ}\text{C}$ after reconstitution, others at 4°C. Keep substrate solution protected from light 2. Stop solution should be added to each well before measure

