

# **Human MIP-1 $\beta$ ELISA Kit**

**User Manual  
(Revised Feb 24, 2008)**

**Human MIP-1 $\beta$   
ELISA Kit Protocol**

## **TABLE OF CONTENTS**

I.	Introduction.....	2
II.	Reagents.....	2
III.	Storage.....	3
IV.	Additional Materials Required.....	3
V.	Reagent Preparation.....	4
VI.	Assay Procedure.....	5
VII.	Assay Procedure Summary.....	6
VIII.	Calculation of Results	
A.	Typical Data.....	7
B.	Sensitivity.....	8
C.	Recovery.....	8
D.	Linearity.....	8
E.	Reproducibility.....	9
IX.	Specificity.....	9
X.	References.....	9
XI.	Troubleshooting Guide.....	10

## I. INTRODUCTION

MIP-1 (macrophage inflammatory protein-1) is an acidic protein. MIP-1 $\alpha$  and MIP-1 $\beta$  have a length of 69 amino acids (7.8 kDa). The two MIP proteins are the major factors produced by macrophages following their stimulation with bacterial endotoxins. Both proteins are involved in the cell activation of human granulocytes and appear to be involved in acute neutrophilic Inflammation. MIP-1 $\beta$  is most effective at augmenting adhesion of CD8 (+) T-cells to the vascular cell adhesion molecule VCAM-1. MIP-1 $\alpha$  and MIP-1 $\beta$  can induce the proliferation and activation of killer cells known as CHAK (CC-Chemokine-activated killer).

The Human MIP-1 $\beta$  ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human MIP-1 $\beta$  in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human MIP-1 $\beta$  coated on a 96-well plate. Standards and samples are pipetted into the wells and MIP-1 $\beta$  present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human MIP-1 $\beta$  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 MIP-1 $\beta$  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. MIP-1 $\beta$  Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human MIP-1 $\beta$ .
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution
3. Standards (Item C): 2 vials of recombinant human MIP-1 $\beta$ .

4. Assay Diluent A (Item D): 30 ml, 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 MIP-1 $\beta$  (Item F): 2 vial of biotinylated anti-human MIP-1 $\beta$  (each vial is enough to assay half microplate)
7. HRP-Streptavidin concentrate (Item G): 8  $\mu$ l 30,000x 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**

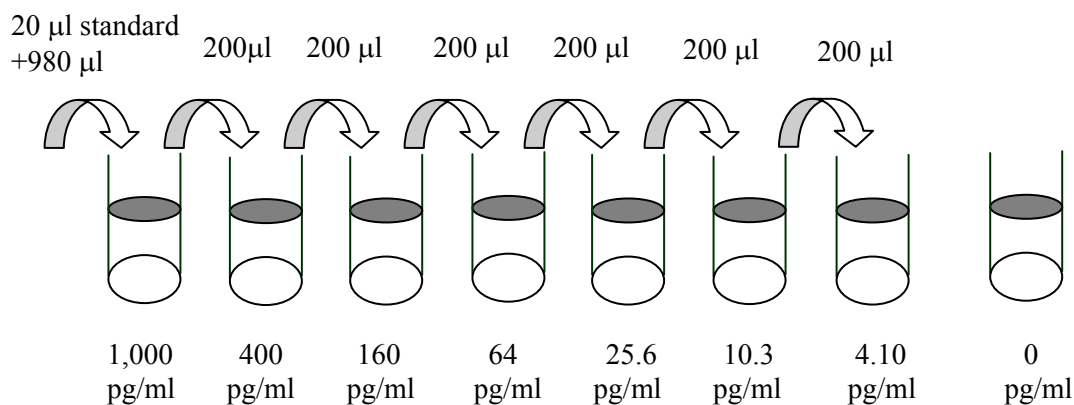
May be stored for up to 5 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20 °C or -80 °C (recommended at -80 °C) after reconstitution. Opened Microplate Wells and reagents may be store for up to 1 month at 2° to 8°C. Return unused wells to the pouch 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  $\mu$ 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.** Add 400  $\mu\text{l}$  Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) into Item C vial to prepare a 50 ng/ml standard. **Dissolve the powder thoroughly by a gentle mix.** Add 20  $\mu\text{l}$  MIP-1 $\beta$  standard from the vial of Item C, into a tube with 980  $\mu\text{l}$  Assay Diluent A or 1x Assay Diluent B to prepare a 1,000 pg/ml stock standard solution. Pipette 300  $\mu\text{l}$  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  $\mu$ 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 30,000-fold with 1x Assay Diluent B.





*For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 2  $\mu$ l of HRP-Streptavidin concentrate into a tube with 198.0  $\mu$ 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 40  $\mu$ l of prepared 100-fold diluted solution into a tube with 12 ml 1x Assay Diluent B to prepare a final 30,000 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  $\mu$ l of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well with plate holder 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  $\mu$ l each).

4. Add 100  $\mu\text{l}$  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  $\mu\text{l}$  each).
6. Add 100  $\mu\text{l}$  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  $\mu\text{l}$  each).
8. Add 100  $\mu\text{l}$  of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark.
9. Add 50  $\mu\text{l}$  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  $\mu\text{l}$  standard or sample to each well.  
Incubate 2.5 hours at room temperature or over night at 4°C.  

3. Add 100  $\mu\text{l}$  prepared biotin antibody to each well.  
Incubate 1 hour at room temperature.  

4. Add 100  $\mu\text{l}$  prepared Streptavidin solution.  
Incubate 45 minutes at room temperature.  


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



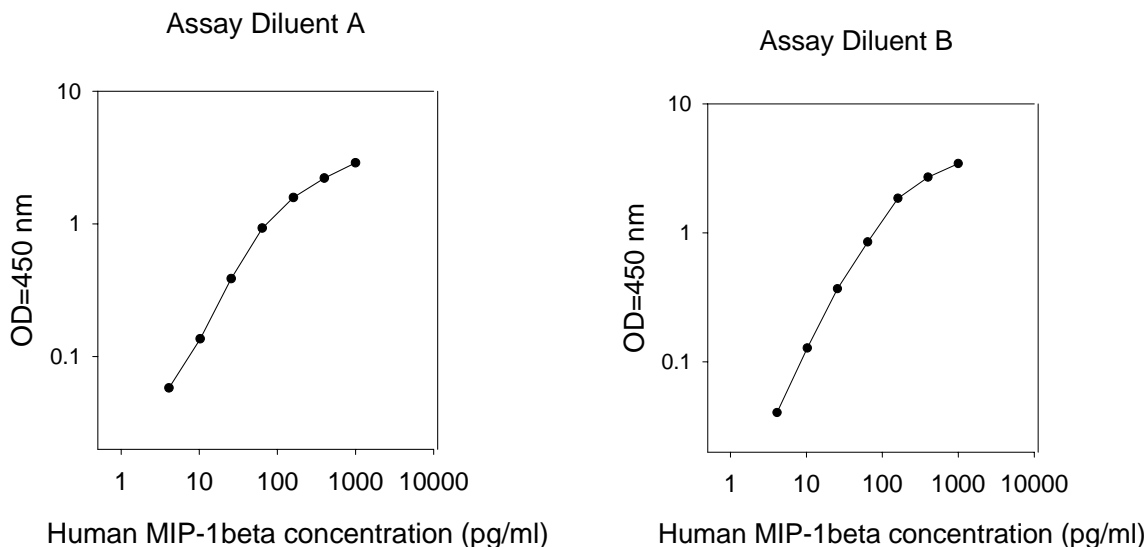
6. Add 50  $\mu$ l Stop Solution to each well.  
Read at 450 nm immediately.

## 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 MIP-1 $\beta$  is typically less than 2.5 pg/ml.

## C. RECOVERY

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

Sample Type	Average % Recovery	Range (%)
Serum	95.64	82-102
Plasma	94.32	83-103
Cell culture media	97.42	85-104

## D. LINEARITY

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected Range (%)	93 83-103	92 82-102	94 83-102
1:4	Average % of Expected Range (%)	96 85-104	95 84-104	102 86-105
1:8	Average % of Expected Range (%)	95 84-103	94 82-102	98 84-103

## **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 $\alpha$ , IL-1 $\beta$ , 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- $\gamma$ , Leptin, MCP-1, MCP-2, MCP-3, MDC, MIP-1 $\alpha$ , MIP-1 $\delta$ , PARC, PDGF, RANTES, SCF, TARC, TGF- $\beta$ , TIMP-1, TIMP-2, TNF- $\alpha$ , TNF- $\beta$ , TPO, VEGF).

## **X. REFERENCES:**

1. Appelberg R, Macrophage inflammatory proteins MIP-1 and MIP-2 are involved in T cell-mediated neutrophil recruitment. *Journal of Leukocyte Biology* 52: 303-306 (1992).
2. Appelberg R, Interferon-gamma (IFN-gamma) and macrophage inflammatory proteins (MIP)-1 and -2 are involved in the regulation of the T cell-dependent chronic peritoneal neutrophilia of mice infected with mycobacteria. *Clinical and Experimental Immunology* 89: 269-273 (1992).
3. Broxmeyer HE et al, Comparative analysis of the human macrophage inflammatory protein family of cytokines (chemokines) on proliferation of human myeloid progenitor cells. Interacting effects involving suppression, synergistic suppression, and blocking of suppression. *Journal of Immunology* 150: 3448-3458 (1993).

## XI. TROUBLESHOOTING GUIDE

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



**Note:**



