

MIP-1 β Sandwich ELISA Kit USER MANUAL

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2.0 INTRODUCTION

The macrophage inflammatory proteins-MIP-1 α and MIP-1 β are members of the β or CC subfamily of chemokines. Both were initially co-purified from the conditional media of a LPS-stimulated mouse macrophage cell line (1). The mature human MIP-1 β and human MIP-1 α share 70% homologous in their protein sequences. MIP-1 β level in synovial fluids is linked to inflammation diseases. Its levels were increased in synovial fluids from patients with osteoarthritis (18.0 +/- 8.9 ng/ml) compared to patients with rheumatoid arthritis (6.1 +/- 2.9 ng/ml) or other forms of arthritis (10.4 +/- 7.0 ng/ml)(2). Determination of the expression levels of the MIP-1s turned out to provide important information regarding numerous diseases such as multiple myeloma, allergic asthmatic disorders, acute experimental autoimmune encephalomyelitis, HIV infection, sarcoidosis and sepsis. The human MIP-1 β ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of MIP-1 β levels in cell culture supernatants, serum and plasma. It is also can detect the recombinant MIP-1 β .

Principle of the MIP-1 β Sandwich ELISA

This assay employs the sandwich enzyme immunoassay technique that detects levels of MIP-1 β . Rabbit anti-MIP-1 β monoclonal antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and MIP-1 β bound to the immobilized antibody. Following extensive washing, biotinylated rabbit anti-MIP-1 β monoclonal antibody reagent is added. Following washes to remove any unbound antibody reagent, streptavidin-HRP is added to the well. After washing away the unbound streptavidin-HRP, a substrate solution is added to the wells to develop color. The magnitude of the absorbance for this developed color is proportional to the amount of MIP-1 β levels.

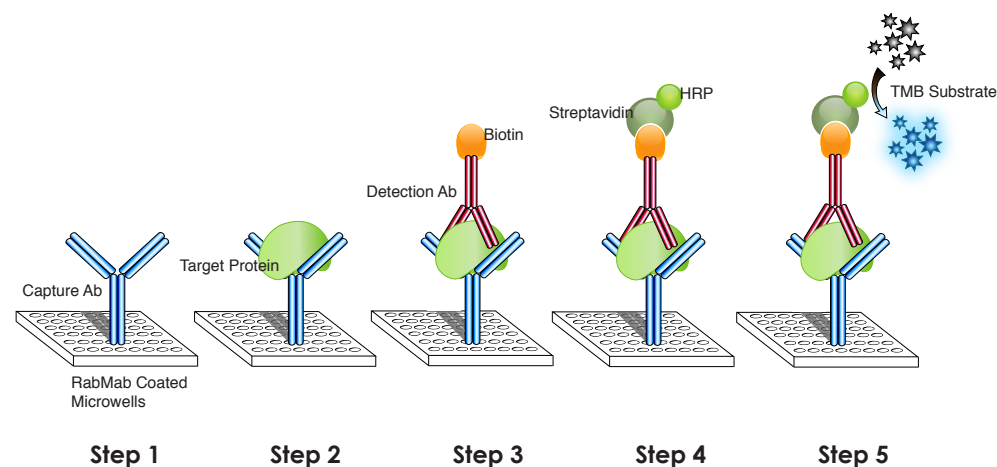


Fig 1. 5 Steps of Sandwich ELISA assay.

3.0 MATERIALS, REAGENTS AND EQUIPMENT

Table 1 – Components/Reagents Provided

Reagent	Quantity	Storage	Color
Anti-MIP-1 β Ab Coated Microwells	96 wells	4 - 8° C	
Biotinylated Anti-MIP-1 β Antibody Reagent (1x)	11 ml	4 - 8° C	
Sample & Standard Diluent CF: (For cell culture supernatants and human plasma)	21 ml	4 - 8° C	
Sample & Standard Diluent GS: (For human serum)	21 ml	4 - 8° C	
Lyophilized recombinant human MIP-1 β standard (2 vials, 4ng/vial)	8ng	-20° C	
Streptavidin-HRP Reagent (100x)	120 ul	4 - 8° C	
Streptavidin-HRP Reagent Diluent Buffer	11 ml	4 - 8° C	
ELISA Washing Buffer (10x)	25 ml	4 - 8° C	blue
TMB A Substrate Solution (1x)	7 ml	4 - 8° C	brown
TMB B Substrate Solution (1x)	7 ml	4 - 8° C	brown

Required Components Not Provided

- Deionized water

Required Equipment Not Provided

- Pipettors and pipet tips
- Vortex mixer or equivalent
- Rotating shaker
- Microtiter plate reader

3.1 NOTES ON MATERIALS

Microtiter Plate

- Bring stripped microtiter plate to room temperature. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate by evenly pushing the bottoms of the microwell strips.
- Store extra strips immediately in the sealed bag at 4°C.






Buffers

- Bring ELISA washing buffer (10x) to room temperature before diluting with Mili-Q or equivalent deionized water.
- Store all buffer and reagents at 4°C when not in use.

4.0 PROCEDURE






(Please read through entire procedure before beginning)

4.1 ELISA Protocol

- 4.1.1 Prepare all reagents and samples as directed in the previous section and bring to room temp.
- 4.1.2 Remove excess microplate strips from plate and return to foil pouch.
- 4.1.3 Reconstitute each lyophilized standard with 1ml of Sample Diluent. Leave the reconstituted standard at room temperature for at least 10 min and mix gently. This reconstitution produces a stock solution of 4000 pg/ml. Use the stock solution to produce a dilution series:
 - 4.1.4 1) Assay with Sample & Standard diluent CF: 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml and CF serves as the zero control (0 pg/ml).
 - 2) Assay with Sample & Standard diluent GS: 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml and GS serves as the zero control (0 pg/ml).
- 4.1.5 * When human serum samples are used, serum must be diluted 3 fold with Sample & Standard diluent GS before testing.
- 4.1.6   Add 100 ul of reconstituted standards, test samples and control in duplicate to each well. Mix well by gentle tapping the plate several times. Carefully cover plate and incubate for 1.5 hours at room temperature on a shaker.
- 4.1.7  Carefully aspirate each well and wash 3 times with 200ul 1x wash buffer per well.
- 4.1.8   Add 100ul of biotinylated anti-human MIP-1 β antibody reagent to each well. Carefully cover plate and incubate for 1 hour at room temperature on a shaker.




 add  incubate  wash

4.1 ELISA Protocol Continued

- 4.1.9  Carefully aspirate each well and wash 3 times with 200ul 1x wash buffer per well.
- 4.1.10  Add 100ul of streptavidin-HRP (1x) reagent to each well. Carefully cover plate and incubate for 30 mins at room temperature on a shaker.
- 4.1.11  Carefully aspirate each well and wash 3 times with 200ul 1x wash buffer per well.
- 4.1.12  Combine TMB A and TMB B (1:1)*
* Volume of each TMB substrate needed = 50 ul (# of wells +1)
Add 100 ul of combined substrate solution to each well. Incubate for 30 min. (max) at room temp. on a shaker.
- 4.1.13  Add 100 ul of Stop Solution to each well.
- 4.1.14 Determine the optical density at 450 nm using a microplate reader within 30 minutes.

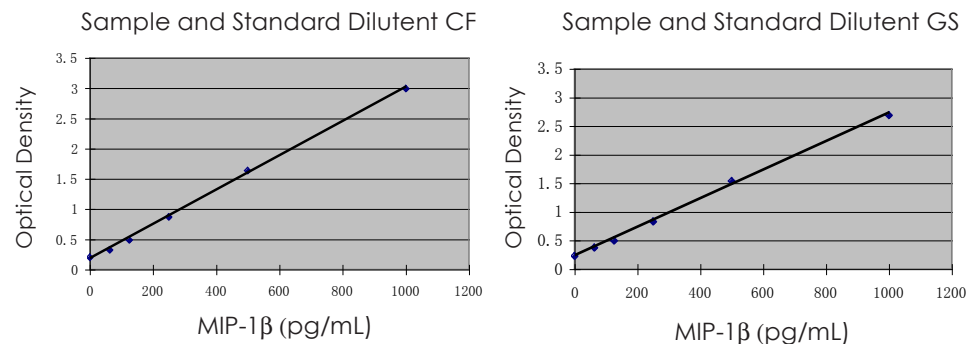
4.2 Calculation of Data

- 4.1.15 Calculate the average absorbance values (A450) for each set of standards and samples.
- 4.1.16 Construct a standard curve by setting the mean absorbance obtained for each standard as Y axis and the concentration in pg/ml as X axis. In excel, fit the curve to get an equation.
- 4.1.17 Using the mean absorbance value for each sample, determine the corresponding concentration of sample in pg/ml from the equation.

 add  incubate  wash

5.0 Typical Data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



5.1 Precision

Cell Culture Supernatant/Plasma

sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
mean (pg/ml)	577.5	136	42.2	484.4	220.9	122.1
standard deviation	17.18	5.39	4.49	28.2	14.3	13.1
CV (%)	2.97	3.96	10.65	5.82	6.48	10.7

Serum

sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
mean (pg/ml)	977	238	100.8	484.4	482.1	237.1
standard deviation	29.6	10.7	10.6	41.4	25.7	16.7
CV (%)	3.03	4.48	10.47	4.60	5.34	7.01

5.2 RECOVERY

The recovery of MIP-1 β spiked to human serum, plasma, and cell culture supernatant was evaluated.

Sample Type	Average % recovery		Range
Serum (n=6)	High:	91.8%	88.5 - 95.2%
	Medium:	83.4 %	80.8 - 86.4%
	Low:	86.1%	78.7 - 90.1%
Plasma (n=6)	High:	100.2%	96.8 - 104.7%
	Medium:	101.6%	97.5 - 106.3%
	Low:	95.1%	91.2 - 99.8%
Cell culture supernatant (n=6)	High:	94.3%	89.5 - 99.9%
	Medium:	97.7%	90.1 - 105.7%
	Low:	102%	87.4 - 110.6%

6.0 SENSITIVITY

When assay with Sample & standard diluent CF, the minimal detection concentration is 43 pg/ml.

When assay with Sample & standard diluent GS, the minimal detection concentration is 30 pg/ml.

6.1 Specificity

This assay recognizes recombinant and natural human MIP-1 β . The antibodies will not recognize MIP-1 α . No significant cross-reactivity or interference was observed.

7.0 REFERENCES

1. Wolpe, S.D. and A. Cerami (1989) FASEB J. 3:2565
2. Koch, A.E. et al. Clin Immunol Immunopathol. 1995 Dec; 77(3):307-14.

8.0 CONTACT

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