

# INSTRUCTIONS

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## Endogen<sup>®</sup> Human MIP-1 $\beta$ ELISA Kit

### EH2MIP1B

1471.1

Number	Description
EH2MIP1B	<b>Human Macrophage Inflammatory Protein-1 beta (MIP-1<math>\beta</math>) ELISA Kit</b> , sufficient reagents for 96 determinations <b>Kit Contents:</b> <b>Anti-Human MIP-1<math>\beta</math> Pre-coated 96-well Strip Plate</b> , 1 each <b>Lyophilized Recombinant Human MIP-1<math>\beta</math> Standard</b> , 2 vials <b>Sample Diluent</b> , 25 ml <b>Biotinylated Antibody Reagent</b> , 7 ml <b>30X Wash Buffer</b> , 50 ml <b>Streptavidin-HRP Reagent</b> , 14 ml <b>TMB Substrate Solution</b> , 13 ml <b>Stop Solution</b> , 13 ml, contains 0.16 M sulfuric acid <b>Adhesive Plate Covers</b> , 6 each

For research use only – not for use in diagnostic procedures.

**Storage:** Upon arrival store all reagents at 2-8°C. Do not freeze reagents. Product shipped with ice pack.

*Refer to the expiration date stamped on the kit box. Do not use kit beyond the stated expiration date.*

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### Introduction

The Endogen<sup>®</sup> Human Macrophage Inflammatory Protein-1 beta (MIP-1 $\beta$ ) ELISA is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human MIP-1 $\beta$  in culture supernatants; EDTA and sodium heparin plasma; and serum.

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**Warranty:** Pierce Biotechnology (hereafter “Pierce”) products are warranted to meet stated product specifications and to conform to label descriptions when stored and used properly. Unless otherwise stated, this warranty is limited to one year from date of sale when used according to product instructions. Pierce’s sole liability for the product is limited to replacement of the product or refund of the purchase price. Unless otherwise expressly authorized in writing by Pierce, products are supplied for research use only and are intended to be used by a technically qualified individual. Pierce’s quality system is certified to ISO 9001. Pierce makes no claim of suitability for use in applications regulated by FDA. Pierce strives for 100% customer satisfaction. If you are not satisfied with the performance of a Pierce product, please contact Pierce or your local distributor.

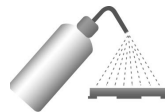
## Procedure Summary



1. Add 50  $\mu$ l of standards or samples to each well in duplicate.\*



2. Cover plate and incubate at room temperature (20-25°C) for 1 hour.



3. Wash plate THREE times.



4. Add 50  $\mu$ l of Biotinylated Antibody Reagent to each well. Cover plate and incubate at room temperature for 1 hour.



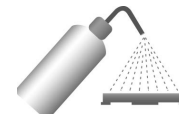
5. Wash plate THREE times.



6. Add 100  $\mu$ l of prepared Streptavidin-HRP Reagent to each well.



7. Cover plate and incubate at room temperature for 30 minutes.



8. Wash plate THREE times.



9. Add 100  $\mu$ l of TMB Substrate Solution to each well.



10. Develop the plate in the dark at room temperature for 10 minutes.



11. Stop reaction by adding 100  $\mu$ l of Stop Solution to each well.



12. Measure absorbance on a plate reader at 450 nm minus 550 nm. Calculate results.

\*Serum and plasma samples must first be diluted 1:1 with Sample Diluent before assaying.

## Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1,000  $\mu$ l
- Plastic pipettes to deliver 5-15 ml
- Ultrapure water
- A glass or plastic two-liter container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5 ml polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoir, 4 each
- A standard ELISA reader for measuring absorbance at 450 nm and 550 nm. If a 550 nm filter is not available, the absorbance can be measured at 450 nm only. Refer to the instruction manual supplied with the instrument being used.
- Graph paper or a computerized curve-fitting statistical software package

## Precautions

- **All samples and reagents must be at room temperature (20-25°C) before use in the assay.**
- Review the instruction booklet carefully and verify all components against the Kit Contents list (page 1) before beginning the assay.
- Do not use a water bath to thaw samples. Thaw samples at room temperature.
- When assaying culture medium, prepare the standard curve and sample dilutions using the same medium used to culture cells. For example, if RPMI with 10% fetal calf serum (FCS) was used to culture cells, then use RPMI with 10% FCS to dilute standard and samples. For best results, use a culture medium that contains a carrier protein such as FCS. Lack of a carrier protein in the media or addition of other compounds to the media may compromise assay results. If alternative media must be used, prepare two standard curves: one with Standard Diluent and one with the alternative media. If the OD values of the two curves are within 10% of the mean for both curves, then the assay may be performed with Standard Diluent. If the OD values of the two curves are not within 10% of the mean for both curves, then perform the assay with the alternative media. If the alternative media has significant effects on the assay, consider using different media.
- If using a multichannel pipettor, always use a new disposable reagent reservoir.
- Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use a new adhesive plate cover for each incubation step.
- Once reagents have been added to the plate, take care NOT to let plate DRY at any time during the assay.
- Avoid microbial contamination of reagents.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Do not mix reagents from different kit lots. Discard unused kit components.
- Do not use glass pipettes to measure TMB Substrate Solution. Take care not to contaminate the solution. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain antibiotics and preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.

## Sample Preparation

### Sample Handling

- Serum; EDTA or sodium heparin plasma; or culture supernatants may be tested in this ELISA.
- For each well, 50  $\mu$ l of serum or plasma diluted 1:1 with Sample Diluent or 50  $\mu$ l per well of culture supernatant is required.
- Store samples to be assayed within 4 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C.
- Avoid repeated freeze-thaw cycles when storing samples.
- Test samples and standards must be assayed in duplicate each time the ELISA is performed.
- Gradually equilibrate samples to room temperature before beginning assay. Do not use heated water baths to thaw or warm samples.
- Mix samples by gently inverting tubes.
- If samples are clotted, grossly hemolyzed, lipemic or microbially contaminated, or if there is any question about the integrity of a sample, make a note on the template and interpret results with caution.

### Sample Dilution

- **Serum and plasma samples must first be diluted 1:1 with Sample Diluent before testing.** To prepare a 1:1 dilution, add 100  $\mu$ l of sample to 100  $\mu$ l of Sample Diluent in a separate tube and mix well. Alternatively, add 25  $\mu$ l of Sample Diluent to the sample wells and then add 25  $\mu$ l of sample to those wells. Tap the plate gently to mix. Either method provides a final 1:1 dilution of the sample in each well.
- If the human MIP-1 $\beta$  concentration possibly exceeds the highest point of the standard curve (i.e., 600 pg/ml), prepare one or more five-fold dilutions of the test sample. When testing **culture supernatants**, prepare the serial dilutions using the culture medium. When testing **serum or plasma**, prepare the serial dilutions using the Sample Diluent provided. For example, a five-fold dilution is prepared by adding 50  $\mu$ l of test sample to 200  $\mu$ l of appropriate diluent. Mix thoroughly between dilutions before assaying.

### Reagent Preparation

For procedural differences when using partial plates, look for **(PP)** throughout this instruction booklet.

### Wash Buffer

1. Label a clean glass or plastic two-liter container "Wash Buffer." The 30X Wash Buffer may have a cloudy appearance.
2. Add the entire contents of the 30X Wash Buffer bottle (50 ml) to the two-liter container and dilute to a final volume of 1.5 liters with ultrapure water. Mix thoroughly.

**(PP)** When using partial plates, store the reconstituted Wash Buffer at 2-8°C.

**Note:** Wash Buffer must be at room temperature before use in the assay. Do not use Wash Buffer if it becomes visibly contaminated during storage.

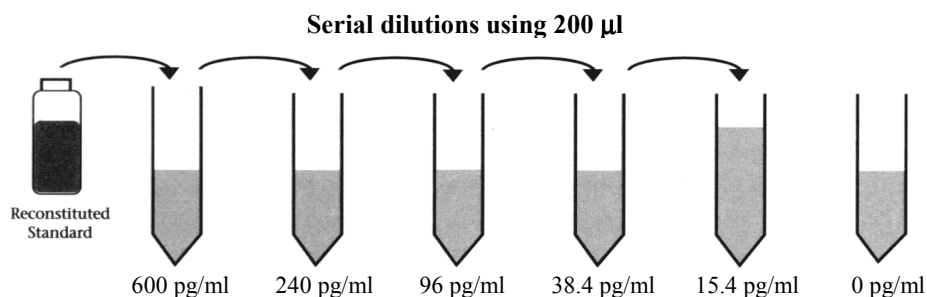
### Standards

- **(PP)** Reconstitute and use one vial of the lyophilized standard per partial plate.
- Prepare standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.
- 1. When testing **culture supernatant samples**, reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting vial. Use the sample culture medium to prepare standard curve serial dilutions.

When testing **serum or plasma samples**, reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting vial. Use the Sample Diluent provided to prepare standard curve serial dilutions.

When testing **serum, plasma and cell culture supernatant samples on the same plate**, validate the medium to establish if the same standard curve can be used for the different sample types. Prepare a standard curve (including a zero) using culture medium to reconstitute and dilute the standard. Use medium containing serum or other protein supplement to maximize stability of the human MIP-1 $\beta$ . Perform this curve in parallel with a standard curve reconstituted in ultrapure water and diluted in the Sample Diluent provided. If the OD values of the two curves are within 10% of the mean for both curves, then the assay can be performed with Sample Diluent, whether testing culture supernatant, plasma or serum samples.

2. Label six tubes, one for each standard curve point: 600 pg/ml, 240 pg/ml, 96 pg/ml, 38.4 pg/ml, 15.4 pg/ml and 0 pg/ml, then prepare 1:2.5 serial dilutions for the standard curve as follows:
3. Pipette 300  $\mu$ l of Standard Diluent into each tube.
4. Pipette 200  $\mu$ l of the reconstituted standard into the first tube (i.e., 600 pg/ml) and mix.
5. Pipette 200  $\mu$ l of this dilution into the second tube (i.e., 240 pg/ml) and mix.
6. Repeat the serial dilutions (using 200  $\mu$ l) three more times to complete the standard curve points. These concentrations, 600 pg/ml, 240 pg/ml, 96 pg/ml, 38.4 pg/ml, 15.4 pg/ml, and 0 pg/ml, are the standard curve points.



## Assay Procedure

### A. Sample Incubation

- **(PP)** Determine the number of strips required. Leave these strips in the plate frame. Place remaining unused strips in the provided foil pouch with desiccant. Make sure foil pouch is sealed tightly. Store reserved strips at 2-8°C. After completing assay, retain plate frame for second partial plate. When using the second partial plate, place strips securely in the plate frame.
  - Use the Data Template provided to record the locations of the zero standard, human MIP-1 $\beta$  standards and test samples. Perform five standard points and one zero in duplicate with each series of unknown samples.
1. Add 50  $\mu$ l of reconstituted standards or test samples in duplicate to each well. Mix well by gently tapping the plate several times.

**Note:** All serum and plasma samples must be diluted 1:1 before testing (see Sample Preparation – Sample Dilution section). If the human MIP-1 $\beta$  concentration in any sample is expected to further exceed the highest point on the standard curve, 600 pg/ml, see Sample Preparation – Sample Dilution section.

2. Carefully cover plate with a new adhesive plate cover. Ensure all edges and strips are sealed tightly by running your thumb over edges and down each strip. Incubate for one (1) hour at room temperature, 20-25°C.
3. Carefully remove adhesive plate cover, discard plate contents and wash plate THREE times with Wash Buffer as described in the Plate Washing section (section B).

### B. Plate Washing

**Note:** Automated plate washers may produce sub-optimal results. For best results, perform a manual wash the first time the assay is performed. Automated plate washing may require validation to determine the number of washes necessary to achieve optimal results; typically, 3-5 washes are sufficient.

1. Gently squeeze the long sides of plate frame before washing to ensure all strips securely remain in the frame.
2. Empty plate contents. Use a squirt wash bottle to vigorously fill each well completely with Wash Buffer, then empty plate contents. Repeat procedure two additional times for a total of THREE washes. Blot plate onto paper towels or other absorbent material.

**Note:** For automated washing, aspirate all wells and wash with Wash Buffer, overfilling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material. The number of washes necessary may require optimization.

### C. Biotinylated Antibody Reagent Incubation

- If using a multichannel pipettor, use a new reagent reservoir and pipette tips when adding the Biotinylated Antibody Reagent. Remove from the vial only the amount required for the number of strips being used.
1. Add 50  $\mu$ l of Biotinylated Antibody Reagent to each well containing sample or standard. Mix well by gently tapping the plate several times.
  2. Carefully attach a new adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over edges and down each strip. Incubate plate for one (1) hour at room temperature, 20-25°C.
  3. Carefully remove the adhesive plate cover, discard plate contents and wash THREE times with Wash Buffer as described in the Plate Washing section (section B).

#### D. Streptavidin-HRP Reagent Incubation

**Note:** If using a multichannel pipettor, **use new reagent reservoir and pipette tips** when adding the Streptavidin-HRP Reagent.

1. Add 100  $\mu$ l of Streptavidin-HRP Reagent to each well.
2. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 30 minutes at room temperature, 20-25°C.
3. Carefully remove the adhesive plate cover, discard plate contents and wash THREE times as described in the Plate Washing section.

#### E. Substrate Incubation and Stop Step

**Note:** If using a multichannel pipettor, **use new reagent reservoir and pipette tips** when adding the TMB Substrate Solution and Stop Solution.

- Dispense from bottle **ONLY** amount required, 100  $\mu$ l per well, for the number of wells being used. Do not use a glass pipette to measure the TMB Substrate Solution.
  - **(PP)** Do not combine leftover substrate with that reserved for the second partial plate. Take care not to contaminate remaining TMB Substrate Solution.
1. Pipette 100  $\mu$ l of TMB Substrate Solution into each well.
  2. Allow enzymatic color reaction to develop at room temperature in the dark for 10 minutes. Do not cover plate with a plate sealer. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
  3. After 10 minutes, stop the reaction by adding 100  $\mu$ l of Stop Solution to each well.

#### F. Absorbance Measurement

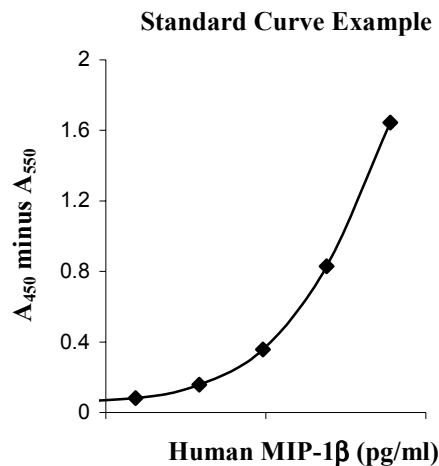
**Note:** Evaluate the plate within 30 minutes of stopping the reaction.

Measure the absorbance on an ELISA plate reader set at 450 nm and 550 nm. Subtract 550 nm values from 450 nm values to correct for optical imperfections in the microplate. If an absorbance at 550 nm is not available, measure the absorbance at 450 nm only.

**Note:** When the 550 nm measurement is omitted, absorbance values will be higher.

#### G. Calculation of Results

- The standard curve is used to determine human MIP-1 $\beta$  amount in an unknown sample. Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding human MIP-1 $\beta$  concentration (pg/ml) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. The human MIP-1 $\beta$  amount in each sample is determined by interpolating from the absorbance value (Y axis) to human MIP-1 $\beta$  concentration (X axis) using the standard curve. For best results, use a four-parameter curve fit as indicated in the standard curve example.
- If the test sample was diluted, multiply the interpolated value obtained from the standard curve by the dilution factor to calculate pg/ml of human MIP-1 $\beta$  in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.



**Performance Characteristics****Sensitivity:** <2 pg/ml

The sensitivity or Lower Limit of Detection (LLD)<sup>1</sup> is determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD.

**Assay Range:** 15.4-600 pg/ml

Suggested standard curve points are 600 pg/ml, 240 pg/ml, 96 pg/ml, 38.4 pg/ml, 15.4 pg/ml, and 0 pg/ml.

**Specificity:**

This ELISA is specific for the measurement of natural and recombinant human MIP-1 $\beta$ . Antibodies used in this ELISA do not cross-react with rhMIP-1 $\delta$ , rhMIP-3 $\alpha$ , rhMIP-3 $\beta$ , rhRANTES, rhGRO $\alpha$ , rhGRO $\beta$ , rhGRO $\gamma$ , rhMCP-1, rmMIP-1 $\alpha$ , rmMIP-1 $\beta$ , rmMIP-1 $\gamma$ , rmMIP-3 $\alpha$ , rmMIP-3 $\beta$ , rrMIP-3 $\alpha$ , rvMIP-I and rvMIP-II. Recombinant human MIP-1 $\alpha$  cross-reacted weakly (<2%).

**Calibration:**

The standard in this ELISA is calibrated to a Endogen Human MIP-1 $\beta$  reference standard.

**Precision:**

Intra-assay CV: <10% (see Table 1)

Inter-assay CV: <10% (see Table 2)

**Table 1.** Intra-assay results using the Endogen Human MIP-1 $\beta$  ELISA Kit.

<u>Mean (pg/ml)</u>	<u>CV%</u>	<u>n</u>
457	3.0	24
167	3.3	24
59	5.5	24

**Table 2.** Inter-assay results using the Endogen Human MIP-1 $\beta$  ELISA Kit.

<u>Mean (pg/ml)</u>	<u>CV%</u>	<u>n</u>
454	2.3	4
170	1.7	4
58	4.5	4

**Recovery:**

Recovery is determined by spiking various levels of recombinant human MIP-1 $\beta$  into serum, sodium heparin plasma and EDTA plasma collected from apparently healthy individuals. Percent recoveries are reported in Table 3.

**Table 3.** Spike and percent recovery values from three sample types.

	<u>Spike Level</u>	<u>Mean Recovery (%)</u>	<u>Range (%)</u>
Serum (n=7)	High	83	57-100
	Medium	89	61-108
	Low	114	65-140
Sodium Heparin (n=8)	High	90	84-100
	Medium	94	84-104
	Low	87	63-107
EDTA (n=8)	High	78	67-90
	Medium	80	60-89
	Low	86	62-116

**Reference**

1. *Immunoassay: A Practical Guide*, Chan and Perlstein, Eds., 1987, Academic Press: New York, p71.

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Data Templates

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												