INSTRUCTIONS

Human MCP-1 ELISA Kit

EH2MCP1 EH2MCP12 EH2MCP15

1572.5

Number	Description
EH2MCP1	**Human MCP-1 ELISA Kit,** sufficient reagents for 96 determinations
EH2MCP12	**Human MCP-1 ELISA Kit,** sufficient reagents for 2 × 96 determinations
EH2MCP15	**Human MCP-1 ELISA Kit,** sufficient reagents for 5 × 96 determinations

Kit Contents	EH2MCP1	EH2MCP12	EH2MCP15
Anti-human MCP-1 Precoated 96-well Strip Plate	1 each	2 each	5 each
Lyophilized Recombinant Human MCP-1 Standard	2 vials	4 vials	10 vials
Standard Diluent, contains 0.1% NaN_3	25mL	2 × 25mL	5 × 25mL
Biotinylated Antibody Reagent, contains 0.1% NaN_3	12mL	2 × 12mL	5 × 12mL
30X Wash Buffer	50mL	2 × 50mL	5 × 50mL
Streptavidin-HRP Concentrate	75µL	2 × 75µL	5 × 75µL
Streptavidin-HRP Dilution Buffer	14mL	2 x 14mL	5 x 14mL
TMB Substrate	13mL	2 × 13mL	5 × 13mL
Stop Solution, contains 0.16M sulfuric acid	13mL	2 × 13mL	5 × 13mL
Adhesive plate sealers	6 each	12 each	30 each

For research use only. Not for use in diagnostic procedures.

Storage: For maximum stability, store in a non-defrosting -20ºC freezer and refer to the expiration date for frozen storage on the label. Alternatively, store at 2-8ºC and refer to the expiration date for refrigerated storage. Once thawed, store at 4ºC until the expiration date for refrigerated storage. Kit is shipped on dry ice.

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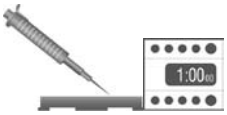
Introduction

The Thermo Scientific™ Human Monocyte Chemoattractant Protein-1 (MCP-1) ELISA is an enzyme-linked immunosorbent assay for quantification of human MCP-1 in serum; EDTA and heparin plasma; and culture supernatant.

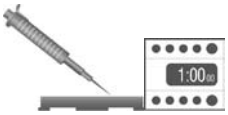
Procedure Summary

1. Add 50µL of Standard Diluent to each well.



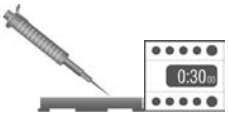
2. Add 50µL of standards or samples in duplicate. Cover plate and incubate at room temperature (20-25°C) for 1 hour.

3. Wash the plate THREE times.



4. Add 100µL of Biotinylated Antibody Reagent to each well. Cover plate and incubate at room temperature for 1 hour.

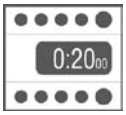
5. Wash the plate THREE times.



6. Add 100µL of prepared Streptavidin-HRP Solution to each well. Cover plate and incubate at room temperature for 30 minutes.

7. Wash the plate THREE times.

8. Add 100µL Premixed TMB Substrate Solution to each well.



9. Develop the plate at room temperature for 20 minutes.

10. Add 100µL of Stop Solution to each well.

11. Measure absorbance. Subtract 550nm values from 450nm values.

12. Calculate results using curve-fitting statistical software.

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1000µL and plastic pipettes to deliver 5-15mL
- 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 reservoirs
- 15mL plastic tube to prepare Streptavidin-HRP Solution
- A standard ELISA reader for measuring absorbance at 450nm and 550nm. If a 550nm filter is not available, the absorbance may be measured at 450nm 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 all instructions carefully and verify components against the Kit Contents list (page 1) before beginning the assay.
- Do not use a heated water bath to thaw samples. Thaw samples at room temperature.
- Sample Diluent must be used when diluting all samples.
- When assaying culture medium, prepare the standard curve and sample dilutions using the same medium used for the cell culture. For example, if RPMI with 10% fetal calf serum (FCS) was used, then use RPMI with 10% FCS to dilute the standard and samples. For best results, use a culture medium that contains a carrier protein such as FCS. The lack of a carrier protein may affect MCP-1 stability and compromise the standard curve. If serum-free media must be used, prepare two standard curves: one with carrier and one without. Compare curves to determine if lack of carrier caused degradation that resulted in a signal decrease.
- If using a multichannel pipettor, always use a new disposable reagent reservoir.
- Use new disposable pipette tips for each transfer and a new adhesive plate cover for each incubation step to avoid cross-contamination.

- 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.
- Avoid exposing reagent 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.
- Some components of this kit contain sodium azide. Please dispose of reagents according to local regulations.

Additional Precaution for the 2-plate and 5-plate Kits

- Dispense, pool, and equilibrate to room temperature only the reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.

Sample Preparation

Sample Handling

- Serum; EDTA or heparin plasma; or culture supernatants may be tested in this assay.
- 50µL per well of serum, plasma or culture supernatant is required.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C. Avoid repeated freeze-thaw cycles when storing samples.
- Gradually equilibrate samples to room temperature before beginning assay. Do not use a heated water bath to thaw or warm samples. Mix samples by gently inverting tubes.
- If samples are clotted, grossly hemolyzed, lipemic or contaminated, or if there is any question about the integrity of a sample, make a note on the template and interpret results with caution.
- Samples and standards must be assayed in duplicate each time the assay is performed.

Sample Dilution

- Culture supernatant samples may be evaluated **undiluted** in this assay. If the human MCP-1 concentration possibly exceeds the highest point of the standard curve (i.e., 1000pg/mL), prepare one or more 10-fold dilutions of the sample using culture media as the diluent. A 10-fold dilution is prepared by adding 50µL of sample to 450µL of culture medium. Mix thoroughly between dilutions.
- Serum and plasma may be evaluated **undiluted** in this assay. If the MCP-1 concentration in a sample possibly exceeds the highest point of the standard curve, (i.e., 1000pg/mL), prepare one or more additional five-fold dilutions. A five-fold dilution is prepared by adding 50µL of sample to 200µL Standard Diluent. Mix thoroughly between dilutions.

Reagent Preparation

For procedural differences when using partial plates, look for **(PP)** throughout these instructions.

Wash Buffer

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.

1. Label a clean glass or plastic 2L container “Wash Buffer.” The 30X Wash Buffer may have a cloudy appearance.
2. Add the entire contents of the 30X Wash Buffer bottle (50mL) to the container and dilute to a final volume of 1.5L with ultrapure water. Mix thoroughly.

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

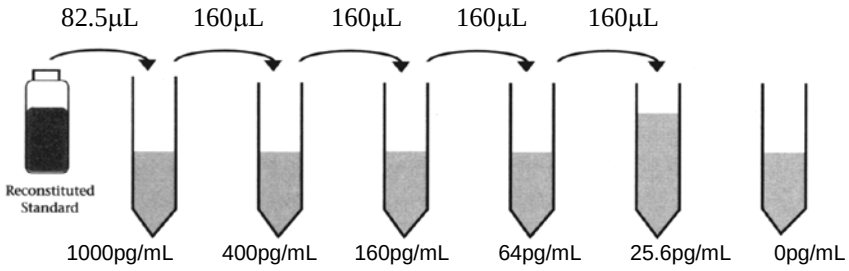
Standards

- **(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 vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting vial. Use 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 vial label. Mix by gently inverting the vial until the contents have completely dissolved. Use the Sample Diluent provided to prepare the standard curve serial dilutions.

   When testing **serum, plasma or 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/blank) using culture medium to dilute the standard. Use medium containing serum or other protein supplement to maximize stability of the MCP1. Perform this curve in parallel with a standard curve diluted in the Standard Diluent provided. If OD values are within 10% of the mean value for both curves, then the assay may be performed with Standard Diluent, whether testing culture supernatant, plasma, urine or serum samples.
2. Label six tubes, one for each standard curve point: 1000pg/mL, 400pg/mL, 160pg/mL, 64pg/mL, 25.6pg/mL and 0pg/mL, then prepare serial dilutions for the standard curve as follows:
3. Pipette 330µL of Standard Diluent into the first tube (i.e., 1000pg/mL)
4. Pipette 240µL of Standard Diluent into the remaining tubes.
5. Pipette 82.5µL of the reconstituted standard into the first tube (i.e., 1000pg/mL) and mix for a 1:5 dilution.
6. Pipette 160µL of this dilution into the next tube (i.e., 400pg/mL) and mix for a 1:2.5 dilution.
7. Repeat the 1:2.5 serial dilutions (using 160µL) three more times to complete the standard curve points.



Assay Procedure

A. Sample Incubation

- **(PP)** Determine the number of strips required and leave these strips in the plate frame. Place remaining unused strips in the provided foil pouch with desiccant and store at 2-8°C. Make sure foil pouch is sealed tightly. 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 locations of the zero standard (blank or negative control), standards and samples. Perform five standard points and one blank in duplicate with each series of unknown samples.

1. Add 50µL of Standard Diluent to each well.
2. Add 50µL of reconstituted standards or samples in duplicate to each well. Mix by gently tapping the plate several times.

   Note: If the human MCP-1 concentration in any sample possibly exceeds the highest point on the standard curve, 1000pg/mL, see Sample Preparation – Sample Dilution Section.
3. 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.
4. Carefully remove adhesive cover, discard plate contents and wash plate as described in the Plate Washing Section below.

B. Plate Washing

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 THREE times with Wash Buffer, overfilling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material.

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 100µL 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 and wash THREE times with Wash Buffer as described in the Plate Washing Section (section B).

D. Streptavidin-HRP Solution Preparation and Incubation

- Prepare Streptavidin-HRP Solution **immediately before use.** Do not prepare more Streptavidin-HRP Solution than required. Do not store prepared Streptavidin-HRP Solution.
- Use a 15mL plastic tube to prepare Streptavidin-HRP Solution.
- **Note:** If using a multichannel pipettor, **use new reagent reservoir and pipette tips** when adding the prepared Streptavidin-HRP Solution.

1. Briefly centrifuge the Streptavidin-HRP Concentrate to force entire vial contents to the bottom.
2. **(PP)** Use only the Streptavidin-HRP Solution amount required for the number of strips being used. For each strip, mix 2.5µL of Streptavidin-HRP Concentrate with 1mL of Streptavidin-HRP Dilution Buffer. Store Streptavidin-HRP Concentrate reserved for additional strips at 2-8°C.

   For one complete 96-well plate, add 30µL of Streptavidin-HRP Concentrate to 12mL of Streptavidin-HRP Dilution Buffer and mix gently.
3. Add 100µL of prepared Streptavidin-HRP Solution to each well.
4. 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.
5. Carefully remove the adhesive cover, discard plate contents and wash plate as described in the Plate Washing Section.

E. Substrate Incubation and Stop Step

- Use new disposable reagent reservoirs when adding TMB Substrate Solution and Stop Solution.
- Dispense from bottle ONLY amount required, 100µL 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.

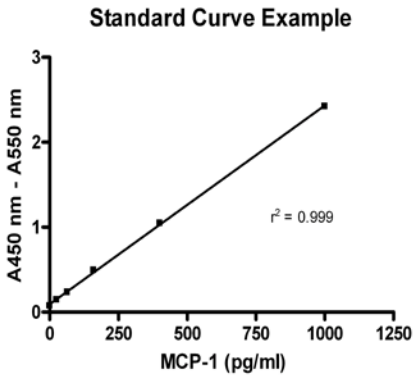
1. Pipette 100µL of TMB Substrate Solution into each well.
2. Allow color reaction to develop at room temperature in the dark for 20 minutes. Do not cover plate with aluminum foil or a plate sealer. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
3. After 20 minutes, stop the reaction by adding 100µL of Stop Solution to each well.

F. Absorbance Measurement

1. Evaluate plate within 30 minutes of stopping the reaction. Measure absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If 550nm is not available, measure absorbance at 450nm only. Omitting the 550nm measurement results in higher absorbance values.

G. Calculation of Results

- Use the standard curve to determine human MCP-1 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 MCP-1 concentration (pg/mL) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software with a linear regression or 4-paramater logistic fit. Determine the human MCP-1 amount in each sample by interpolating from the absorbance value (Y axis) to MCP-1 concentration (X axis) using the standard curve.
- If the sample was diluted, multiply the obtained interpolated value by the dilution factor to calculate amount of MCP-1 in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicates with values that differ from the mean by greater than 10%.



Performance Characteristics

Sensitivity: < 10pg/mL
The sensitivity or lower limit of detection (LLD)[1] 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. This value is the smallest dose that is not zero with 95% confidence.

Assay Range: 0-1000pg/mL
Suggested standard curve points are 1000pg/mL, 400pg/mL, 160pg/mL, 64pg/mL, 25.6pg/mL, and 0pg/mL.

Reproducibility:
Intra-assay CV: <10%
Inter-assay CV: <10%

Specificity: This ELISA is specific for the measurement of natural and recombinant human MCP-1. This ELISA does not cross-react with the following recombinant human chemokines and cytokines: Angiogenin, EGF, FGF-basic, Eotaxin, GM-CSF, GROα, HB-EGF, HGF, IFNα, IFNγ, KGF, MCP-2, MCP-3, MCP-4, MIP-1α, MIP-1β, PLGF-1, RANTES, TNFα, IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, and IL-17.
Calibration: The standards in this ELISA are calibrated to the NIBSC recombinant MCP-1 standard 92/794. One (1) pg of internal standard = 0.58pg of NIBSC standard = 0.00058 NIBSC units.

Expected Values: Serum and plasma samples were collected from apparently healthy human donors and tested in this assay. The levels of human MCP-1 found in each sample type are reported in Table 1.

Table 1. Human MCP-1 levels from apparently healthy individuals.

Sample Type	Average	Range
Serum (n= 15)	305pg/mL	228 - 475pg/mL
EDTA Plasma (n=14)	117pg/mL	58 - 234pg/mL
Heparin Plasma (n=14)	278pg/mL	163 - 438pg/mL

Spike and Recovery: Recovery was determined by spiking various levels of recombinant human MCP-1 into serum and plasma samples collected from apparently healthy individuals and a Standard Diluent control buffer. Mean recoveries are reported in Table 2.

Table 2. Human MCP-1 percent recovery from different sample types.

	Spike Level (pg/mL)		
Sample	**85**	**268**	**500**
Serum (n=5)	82%	77%	84%
EDTA Plasma (n=5)	79%	85%	88%
Heparin Plasma (n = 5)	108%	100%	101%

Linearity: To determine assay linearity, five samples were spiked with a known value of recombinant MCP-1 in serum, EDTA plasma, and heparin plasma. Each sample was serially diluted with standard diluent to produce values that are within the assay range (Table 3).

Table 3. Assay linearity from different sample types.

Dilution		Serum	EDTA plasma	Heparin plasma
1:2	Average % of Expected	88	97	91
	Range (%)	82 - 95	93-103	79-99
1:4	Average % of Expected	93	110	115
	Range (%)	84 - 97	104-114	112-120
1:8	Average % of Expected	86	110	90
	Range (%)	83 - 116	106-118	83-97

Cited Reference

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

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												
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