INSTRUCTIONS

Rat MCP-1 ELISA Kit

ERMCP1 ERMCP12 ERMCP15

1366.5

Number	Description
ERMCP1	**Rat Monocyte Chemoattractant Protein-1 (MCP-1) ELISA Kit,** sufficient reagents for 96 determinations
ERMCP12	**Rat Monocyte Chemoattractant Protein-1 (MCP-1) ELISA Kit,** sufficient reagents for 2 × 96 determinations
ERMCP15	**Rat Monocyte Chemoattractant Protein-1 (MCP-1) ELISA Kit,** sufficient reagents for 5 × 96 determinations

Kit Contents	ERMCP1	ERMCP12	ERMCP15
Anti-Rat MCP-1 Precoated 96-well Strip Plate	1 each	2 each	5 each
Lyophilized Recombinant Rat 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	8mL	2 × 8mL	5 × 8mL
30X Wash Buffer	50mL	2 × 50mL	5 × 50mL
Streptavidin-HRP Concentrate	75µL	2 × 75µL	5 × 75µL
Streptavidin-HRP Dilution Buffer	14mL	2 × 14mL	5 × 14mL
TMB Substrate	13mL	2 × 13mL	5 × 13mL
Stop Solution, contains 0.16M sulfuric acid	13mL	2 × 13mL	5 × 13mL
Adhesive plate covers	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.

Table of Contents

Introduction

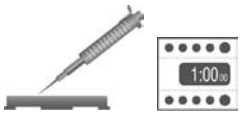
The Thermo Scientific™ Rat MCP-1 ELISA Kit is for measuring rat MCP-1 in culture supernatants; EDTA, sodium citrate and heparin plasma; and serum.

Procedure Summary

1. Add 50µL of standards or diluted 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 50µL of Biotinylated Antibody Reagent to each well. Cover plate and incubate at room temperature for 1 hour.

5. Wash plate THREE times.

6. Add 100µL of prepared Streptavidin-HRP Solution to each well.

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

8. Wash plate THREE times.

9. Add 100µL of TMB Substrate to each well.

10. Develop plate in the dark at room temperature for 30 minutes.

11. Stop reaction by adding 100µL of Stop Solution to each well.

12. Measure absorbance on a plate reader at 450nm minus 550nm. Calculate results using graph paper or 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 2L container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5mL 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
- Microcentrifuge 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 can 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. Do not use a water bath to thaw samples. Thaw samples at room temperature.
- Review all instructions carefully and verify components against the Kit Contents list (page 1) before beginning the assay.
- When preparing standard curve and sample dilution in culture medium, use the same medium used to culture the cells. For example, if RPMI with 10% fetal calf serum (FCS) was used to culture cells, then use RPMI with 10% FCS to dilute the standard and samples. Do NOT use RPMI without serum supplement.
- To avoid cross-contamination, always use a new disposable reagent reservoir, new disposable pipette tips for each transfer and 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.
- 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, heparin and sodium citrate plasma; or culture supernatants may be tested in this ELISA.
- Dilute **serum or plasma** samples 1:25 and use 50µL per well. For **culture supernatant** samples**,** use 50µL per well. Culture supernatant samples at concentrations < 1500pg/mL do not require dilution.
- 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.
- Always assay samples and standards in duplicate each time the ELISA is performed.
- 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 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

- **Culture supernatant samples** may be assayed neat (undiluted). Endogen scientists have observed up to 70ng/mL of MCP-1 in culture supernatants from stimulated rat cells. If the rat MCP-1 concentration of a culture supernatant sample possibly exceeds the highest point of the standard curve (i.e., 1500pg/mL), prepare one or more ten-fold dilutions using culture medium. For example, prepare a ten-fold dilution by adding 50µL of sample to 450µL of culture medium. Mix thoroughly between dilutions before assaying.
- Always dilute **Serum and plasma samples** 1:25 in Standard Diluent before assaying by adding 10µL of serum or plasma to 240µL of Standard Diluent and mix well. If the rat MCP-1 concentration of a test sample diluted 1:25 possibly exceeds the highest point of the standard curve (i.e., 1500pg/mL), prepare one or more additional five-fold dilutions. For example, prepare a five-fold dilution by adding 50µL of diluted sample to 200µL of Standard Diluent. Mix thoroughly between dilutions before assaying.

Reagent Preparation

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

Wash Buffer

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 2L 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.

   Note: Wash Buffer must be at room temperature before use in the assay. Do not use Wash Buffer that has become 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 to the volume indicated 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 to the volume indicated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting vial. Use the Standard Diluent provided to prepare standard curve serial dilutions.

   When testing **serum or 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/blank) using culture medium to reconstitute and dilute the standard. Use medium containing serum or other protein to maximize stability of the rat MCP-1. Perform this curve in parallel with a standard curve reconstituted in ultrapure water and diluted in the Standard Diluent provided. If both curves have OD values within 10% of the mean, then perform the assay with Standard Diluent, whether testing culture supernatant, serum or plasma samples.

2. Label six tubes, one for each standard curve point: 1500, 600, 240, 96, 38, and 0pg/mL, then prepare 1:2.5 serial dilutions for the standard curve as follows:

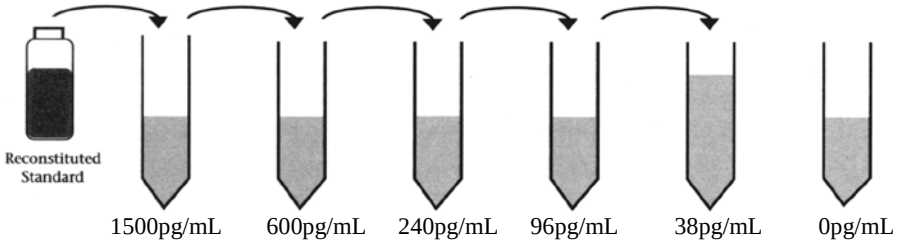
3. Pipette 240µL of appropriate diluent into each tube.

4. Pipette 160µL of the reconstituted standard into the first tube (i.e., 1500pg/mL) and mix.

5. Pipette 160µL of this dilution into the second tube (i.e., 600pg/mL) and mix.

6. Repeat serial dilutions (using 160µL) three more times to complete the standard curve points.

Serial dilutions using 160µL



Assay Procedure

A. Sample Incubation

- **(PP)** Determine the number of strips required. Leave these strips in the plate frame. Tightly seal remaining unused strips in the provided foil pouch with desiccant and store at 2-8°C. After completing the 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 (blank or negative control), rat MCP-1 standards and test samples. Perform six standard points and one blank in duplicate with each series of unknown samples.

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

   Note: Serum and plasma samples must be diluted 1:25 before use in this assay. If the rat MCP-1 concentration in any sample possibly exceeds the highest point on the standard curve, 1500pg/mL, see the Sample Preparation – Sample Dilution Section.

2. Add 50µL of Standard Diluent to all wells that do not contain standards or samples.
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 1 hour at room temperature, 20-25°C.
4. Carefully remove adhesive plate cover. 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. Take care not to touch the samples in wells with the pipette tip when adding the Biotinylated Antibody Reagent.

1. Add 50µL of Biotinylated Antibody Reagent to each well containing sample or standard.
2. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 1 hour 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 (Section B).

D. Streptavidin-HRP Solution Preparation and Incubation

- Prepare Streptavidin-HRP Solution immediately before use. Do not prepare more solution than required.
- Use a 15mL plastic tube to prepare Streptavidin-HRP Solution.
- **Note:** If using a multichannel pipettor, use a 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 plate cover, discard plate contents and wash THREE times as described in the Plate Washing Section.

E. Substrate Incubation and Stop Step

- Use new disposable reagent reservoirs when adding TMB Substrate 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.

1. Pipette 100µL of TMB Substrate into each well.
2. Allow enzymatic reaction to develop at room temperature in the dark for 30 minutes. Do not cover plate. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
3. After 30 minutes, stop the reaction by adding 100µL of Stop Solution to each well.

F. Absorbance Measurement

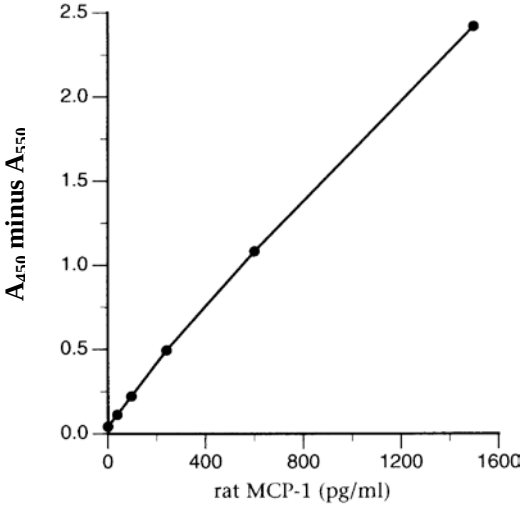
Note: Evaluate the plate within 30 minutes of stopping the reaction.

Measure the absorbance on a plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If a 550nm measurement is not possible, measure absorbance at 450nm only. When the 550nm measurement is omitted, absorbance values will be higher.

G. Calculation of Results

- The standard curve is used to determine rat 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 rat MCP-1 concentration (pg/mL) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the rat MCP-1 amount in each sample by interpolating from the absorbance value (Y-axis) to rat MCP-1 concentration (X-axis) using the standard curve.
- For **Serum and plasma samples** multiply the value interpolated from the standard curve by 25 to calculate the pg/mL of rat MCP-1. If the sample was further diluted, multiply the interpolated value by the total dilution factor. For undiluted samples, such as **culture supernatant**, interpolate the pg/ml of rat MCP-1 directly from the standard curve.
- 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%.

Standard Curve Example



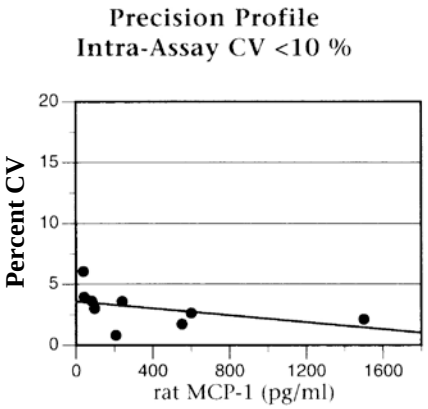
Performance Characteristics

Sensitivity: ≤ 5pg/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: 38-1500pg/mL
Suggested standard curve points are 1500, 600, 240, 96, 38 and 0pg/mL.

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

Specificity: This ELISA is specific for the measurement of natural and recombinant rat MCP-1. This ELISA does not cross-react or interfere with the following chemokines and cytokines: rat IFNγ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, MIP-1α, RANTES, mouse MCP-1, or human MCP-1.



Calibration: The standard in this ELISA is calibrated to an Endogen rat MCP-1 reference standard.

Precision: The intra-assay coefficient of variation is plotted against MCP-1 concentration (pg/mL). The points represent samples evaluated in replicates of four in four different kit lots.

Expected Values: Serum and plasma samples collected from apparently healthy rats are evaluated in this assay (Table 1).

Table 1. Rat MCP-1 levels detected in serum and plasma samples collected from apparently healthy rats.

Sample Type	Average (pg/mL)	Range (pg/mL)
Serum (n=10)	17,306	16,023-20,302
EDTA Plasma (n=6)	3894	2392-5783
Citrate Plasma (n=5)	4330	3473-5506
Heparin Plasma (n=6)	5385	3568-10,380

Recovery: Recovery across the range of the standard curve is determined by spiking various levels of recombinant rat MCP-1 into serum and plasma samples collected from apparently healthy rats and a Standard Diluent control buffer (Table 2). Endogenous levels of rat MCP-1 present in the samples are subtracted and recovery is calculated as a percentage of the Standard Diluent control.

Table 2. Average percent recovery from spiking recombinant rat MCP-1 into serum and plasma samples collected from apparently healthy rats.

	Spike Level		
Sample Type	1000 (pg/mL)	500 (pg/mL)	250 (pg/mL)
Serum (n=3)	91%	97%	104%
EDTA Plasma (n=3)	101%	106%	99%
Heparin Plasma (n=3)	92%	97%	111%
Citrate Plasma (n=3)	89%	97%	112%

Dilution Linearity: Serum, plasma and culture supernatant samples containing rat MCP-1 are serially diluted in the appropriate diluent and evaluated in the rat MCP-1 ELISA. Observed values are compared to expected values and the percent recovery is calculated to assess the dilution linearity of the assay (Table 3).

Table 3. Dilution linearity of the Rat MCP-1 ELISA Kit.

Sample	Dilution	Expected Recovery (%)	Range (%)
Serum (n=2)	1:2	101	100-103
	1:4	98	95-101
	1:8	98	95-101
Plasma (n=4)	1:2	100	97-103
	1:4	90	83-97
	1:8	92	81-98
Culture Supernatant (n=4)	1:2	102	99-106
	1:4	96	86-106
	1:8	101	95-108

Cited Reference

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

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Data Templates

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B												
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