# Mouse MCP-1 ELISA Kit

For the quantitative determination of mouse monocyte chemoattractant protein-1 (MCP-1) concentrations in mouse serum, cell culture supernatant, and other biological fluids

Catalogue Number: MEC1005

*96 tests*

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (07/13-02) mMCP-1

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## INTENDED USE

This Mouse MCP-1 ELISA kit is to be used for the *in vitro* quantitative determination of mouse Monocyte Chemoattractant Protein-1 (MCP-1) concentrations in serum, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY.

## INTRODUCTION

Monocyte chemotactic protein 1(MCP-1), also known as monocyte chemotactic and activating factor(MCAF), lymphocyte–derived chemotactic factor (LDCF) and glioma-derived chemotactic factor (GDF), is a chemotactic cytokine for monocytes. It is a CC chemokine with two adjacent cystines at its NH2 terminus. MCP1 is produced by a variety of cell types, including monocytes, lymphocytes, fibroblasts, endothelial cells, epithelial cells and smooth muscle cells. MCP-1 is up-regulated in response to infectious agents, oxidative radicals and pro-inflammatory mediators released by host cells.

The major physiological function of MCP-1 is to mediate host defense. MCP-1 acts as a chemoattractant to recruit monocytes to the infected or injured area and activates the cells to secret cytokines and superoxide, enhances phagocytosis and antigen presentation by macrophages.

The expression patterns of MCP-1 and IL-8 are similar. Both cytokines will increase upon stimulation with LPS and inflammatory cytokines such as IL-1, TNF-$\alpha$, and IFN-$\gamma$. In mouse fibroblasts, platelet derived growth factor (PDGF) is a strong inducer of MCP-1 mRNA expression but failed to induce IL-8 mRNA, suggesting different regulatory mechanisms.

In addition to being chemotactic for monocytes, MCP-1 also activates mouse monocytes to become cytostatic for several mouse tumour cell lines, release lysosomal enzymes, and generate superoxide.

Elevated MCP-1 levels are associated with autoimmune disease, allergic inflammation, atherosclerosis, glomerulonephritis, granuloma formation. This ELISA kit provides a tool for studying MCP-1 expression and its relationship with various diseases in animal model.

## PRINCIPLE OF THE ASSAY

This mouse MCP-1 enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for mouse MCP-1. Standards or samples are then added to the appropriate microtiter plate wells and incubated. Mouse MCP-1, if present, will bind and become immobilized by the antibody pre-coated on the wells. A biotin conjugated antibody specific for mouse MCP-1 is added to each well. The biotin conjugated antibody will bind to the mouse MCP-1 on the plate. The microtiter plate wells are thoroughly washed to remove unbound biotin conjugate and other components of the sample. Avidin has a very high affinity to biotin. In order to quantify the amount of mouse MCP-1 present in the sample, a standardized preparation of avidin conjugated horseradish peroxidase (HRP) is added to each well. Avidin-HRP will bind to the biotin on plate during incubation. The wells are thoroughly washed to remove all unbound Avidin-HRP conjugate and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only wells to which mouse MCP-1, Biotin conjugate and Avidin-HRP are attached will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of mouse MCP-1 in the samples, this kit contains two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant/ urine testing). According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D.) versus MCP-1 concentration (pg/mL). The concentration of MCP-1 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

*This MCP-1 ELISA is a 3.5-hour solid-phase immunoassay readily applicable to measure MCP-1 levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 1000pg/mL.*

**REAGENTS PROVIDED**
All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

| | ***96 tests*** |
|---|---|

1. **MCP-1 MICROTITER PLATE** (Part MEC05-1) ***96 wells***
   Pre-coated with anti-mouse MCP-1 monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part MEC05-2) ***6 mL***
   Anti-mouse MCP-1 monoclonal antibody conjugated to Biotin.

3. **AVIDIN HRP CONJUGATE** (Part MEC05-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase

4. **MCP-1 STANDARD** (Part MEC05-4) ***2 vials***
   Recombinant mouse MCP-1 (1000 pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part MEC05-5) ***25 mL***
   Animal serum with preservative. *For serum testing*.

6. **CALIBRATOR DILUENT II** (Part MEC05-6) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant/urine testing.*

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part MEC05-7) ***10 mL***
   Buffered solution with $H_2O_2$.

9. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.

10. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid ($H_2SO_4$). Caution: Caustic Material!

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100μL and 50-200μL required for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

## PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Mouse serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from mouse blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate mouse viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat and flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

## SAMPLE PREPARATION

### 1. COLLECTION, HANDLING, AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum and cell culture supernatant are to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay, slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

## PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

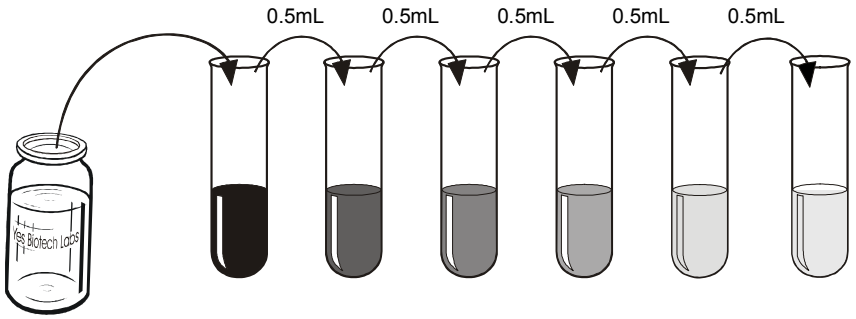
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

| Strips Used | Substrate A (mL) | Substrate B (mL) | Substrate Solution (mL) |
|---|---|---|---|
| 2 strips (16 wells) | 1.5 | 1.5 | 3.0 |
| 4 strips (32 wells) | 3.0 | 3.0 | 6.0 |
| 6 strips (48 wells) | 4.0 | 4.0 | 8.0 |
| 8 strips (64 wells) | 5.0 | 5.0 | 10.0 |
| 10 strips (80 wells) | 6.0 | 6.0 | 12.0 |
| 12 strips (96 wells) | 7.0 | 7.0 | 14.0 |

3. **MCP-1 Standard:**

a) Two vials of Standards are provided in this kit to allow both serum and cell culture supernatant testing. Reconstitute the mouse MCP-1 Standard with either 1.0mL of Calibrator Diluent I (for serum testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 1000pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The mouse MCP-1 standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles: aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (15.6pg/mL to 1000pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted MCP-1 Standard will serve as the high standard (1000pg/mL) and the Calibrator Diluent will serve as the zero-standard (0pg/mL).




1000pg/mL 500pg/mL 250pg/mL 125pg/mL 62.5pg/mL 31.25pg/mL 15.6pg/mL

## ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and mouse MCP-1 Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

| **Wells** | Contents | | **Wells** | Contents | |
|---|---|---|---|---|---|
| **1A, 1B** | Standard 1 – **0 pg/mL** | (S1) | **2A, 2B** | Standard 5 – **125 pg/mL** | (S5) |
| **1C, 1D** | Standard 2 – **15.6 pg/mL** | (S2) | **2C, 2D** | Standard 6 – **250 pg/mL** | (S6) |
| **1E, 1F** | Standard 3 – **31.2 pg/mL** | (S3) | **2E, 2F** | Standard 7 – **500 pg/mL** | (S7) |
| **1G, 1H** | Standard 4 – **62.5 pg/mL** | (S4) | **2G,2H** | Standard 8 -**1000 pg/mL** | (S8) |
| | | | **3A-12H** | MCP-1 samples | |

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | S1 | S5 | 2 | 6 | 10 | 14 | 18 | 22 | 26 | 30 | 34 | 38 |
| **B** | S1 | S5 | 2 | 6 | 10 | 14 | 18 | 22 | 26 | 30 | 34 | 38 |
| **C** | S2 | S6 | 3 | 7 | 11 | 15 | 19 | 23 | 27 | 31 | 35 | 39 |
| **D** | S2 | S6 | 3 | 7 | 11 | 15 | 19 | 23 | 27 | 31 | 35 | 39 |
| **E** | S3 | S7 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 |
| **F** | S3 | S7 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 |
| **G** | S4 | S8 | 5 | 9 | 13 | 17 | 21 | 25 | 29 | 33 | 37 | 41 |
| **H** | S4 | S8 | 5 | 9 | 13 | 17 | 21 | 25 | 29 | 33 | 37 | 41 |

2. Add 50µL of Standard or sample to the appropriate well of the antibody pre-coated Microtiter Plate. Cover and incubate for 1 hour at room temperature.

3. Without discarding the standards and samples, add 50µL Anti-MCP-1 Biotin conjugate to each wells. Mix the contents in wells by placing the plate on a mixer for 1 min at 60RPM, or by tapping gently at the side of the plate for 1 min. Make sure that the contents in the wells is not spilled over and/or contaminated. Cover plate and incubate for 1 hour at room temperature.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Dispense 100μL of conjugate to each well. Cover and incubate for 1 hour at room temperature.

6. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

7. Repeat wash procedure as described in Step 4.

8. Add 100μL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

9. Add 100μL Stop Solution to each well. Mix well.

10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

**CALCULATION RESULT**

The standard curve is used to determine the amount of mouse MCP-1 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding MCP-1 concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of MCP-1 in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding MCP-1 concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

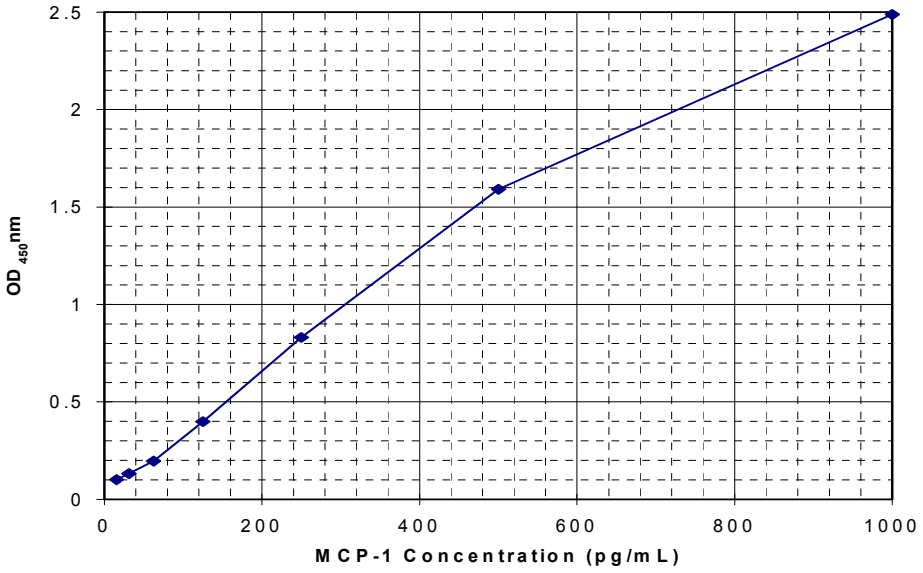
## TYPICAL DATA

Results of a typical standard run of a mouse MCP-1 ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate user results.

### EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

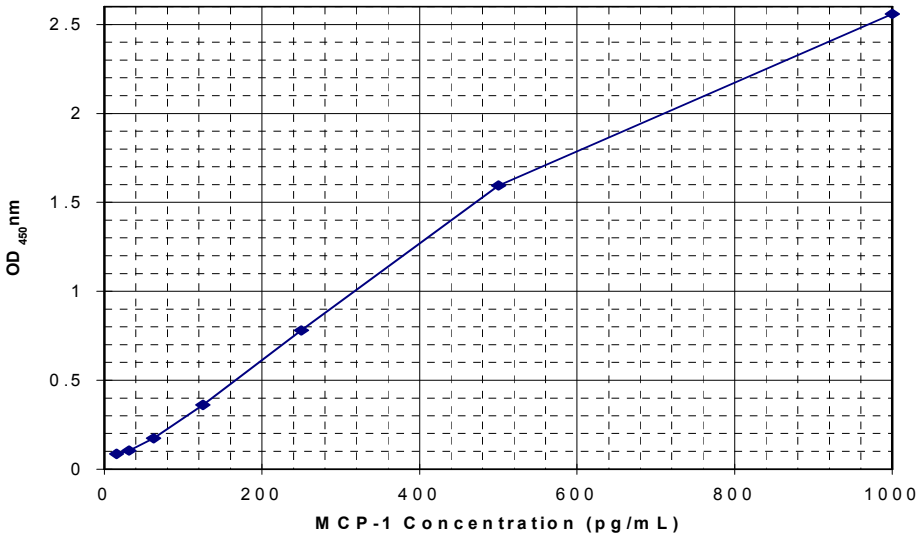
| **Standard (pg/mL)** | **Mean O.D. (450 nm)** | **%CV** | **Zero Standard Subtracted** (Std.)-(S1) |
|---|---|---|---|
| 0 | 0.0600 | 2.24 | 0 |
| 15.6 | 0.1010 | 4.20 | 0.0410 |
| 31.2 | 0.1320 | 0.13 | 0.0720 |
| 62.5 | 0.1965 | 2.52 | 0.1365 |
| 125 | 0.3995 | 4.07 | 0.3395 |
| 250 | 0.8310 | 2.55 | 0.7710 |
| 500 | 1.5910 | 1.16 | 1.5410 |
| 1000 | 2.4880 | 3.13 | 2.4280 |



## EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

| Standard (pg/mL) | Mean O.D. (450 nm) | %CV | Zero Standard Subtracted (Std.)-(S1) |
|---|---|---|---|
| 0 | 0.0515 | 9.61 | 0 |
| 15.6 | 0.0860 | 0.00 | 0.0345 |
| 31.2 | 0.1055 | 2.01 | 0.0540 |
| 62.5 | 0.1745 | 0.81 | 0.1230 |
| 125 | 0.3615 | 4.11 | 0.3100 |
| 250 | 0.7795 | 2.27 | 0.7280 |
| 500 | 1.5950 | 0.53 | 1.5435 |
| 1000 | 2.5595 | 0.36 | 2.5080 |




## PERFORMANCE CHARACTERISTICS

### 1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by using 8 replicates in 1 assay.

| Sample | 1 | 2 | 3 |
|---|---|---|---|
| N | 8 | 8 | 8 |
| Mean (pg/ml) | 245 | 62.5 | 15.6 |
| Coefficient of Variation (%) | 5.54 | 2.61 | 5.23 |

**2. INTER-ASSAY PRECISION**

To determine between-run precision, three different samples of known concentration were assayed by using replicates on 18 different assays.

| | Calibrator Diluent II assay | | |
|---|---|---|---|
| **Sample** | **1** | **2** | **3** |
| N | 18 | 18 | 18 |
| Mean (pg/mL) | 252.4 | 57.86 | 13.36 |
| *Coefficient of Variation (%)* | 2.33 | 2.99 | 6.44 |

**3. RECOVERY**

The recovery of mouse MCP-1 in cell culture media and mouse serum was evaluated with mouse MCP-1 spiked samples.

| Sample Type | Average Recovery % | Range % |
|---|---|---|
| Cell culture media | 97 | 91 -103 |
| Mouse Serum | 75% | 70-80 |

**4. SENSITIVITY**

The minimum detectable dose of mouse MCP1 was determined by adding two standard deviations to the mean optical density value of 16 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of mouse MCP1calculated from calibrate I diluted standard curve was <3.4pg/mL.

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