CELL APPLICATIONS, INC.

# Human MCP-1 ELISA Kit

Cat. No. CL0441
96-wells

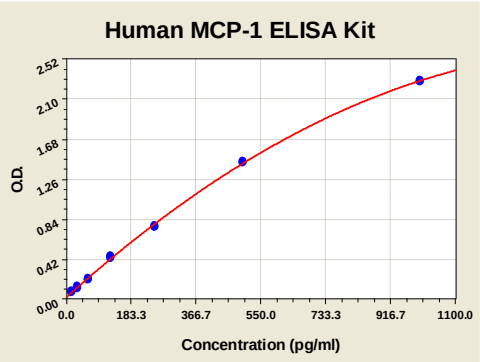
| | |
|---|---|
| **Target Protein Species:** | Human |
| **Range** | 15.6 pg/ml – 1000pg/ml |
| **Specificity** | Cross-reactivates with human Eotaxin, MCP-3 <1%. |

## KIT COMPONENTS

| Component | Amount |
|---|---|
| 96-well plate precoated with anti-human MCP-1 antibody | 1 Plate |
| Protein Standard: Lyophilized recombinant human MCP-1 | 2 tubes, 10 ng/tube |
| Sample Diluent Buffer | 30 ml |
| Biotinylated Antibody (Anti-human MCP-1) | 130 µl (100x) |
| Antibody Diluent Buffer | 12ml |
| Avidin-Biotin-Peroxidase Complex (ABC) Solution | 130 µl (100x) |
| ABC Diluent Buffer | 12 ml |
| Tetramethyllbenzidine (TMB) Color Developing Agent | 10 ml |
| TMB Stop Solution | 10 ml |

### Storage

Store at 4°C. Cell Applications, Inc. recommends using the kit within 6 months of order.




| X | pg/ml | 0.0 | 15.6 | 31.3 | 62.5 | 125 | 250 | 500 | 1000 |
|---|---|---|---|---|---|---|---|---|---|
| Y | O.D.450 | 0.014 | 0.077 | 0.132 | 0.215 | 0.451 | 0.761 | 1.438 | 2.288 |

**Figure 1:** MCP-1 **Standard Curve.** Using the human MCP-1 ELISA Kit, O.D. data was graphed against MCP-1 protein concentration. The TMB reaction was incubated at 37° C for 16 min.

## BACKGROUND

Monocyte chemoattractant protein-1 (MCP-1) was the first CC chemokine identified. Human MCP-1 is composed of 76 amino acids and is 13 kDa in size. MCP subfamily composed of at least four members (MCP-1, -2, -3, and -4).[1] MCP-1 is produced by a variety of cell types, either constitutively or after induction by oxidative stress, cytokines, or growth factors. Many activities have been assigned to MCP-1, including induction of migration of macrophages/monocytes, memory/activated T cells and NK cells and activation of mast cells. MCP-1 mediates its cellular effects primarily through its binding to CCR2, which exists in A and B forms that arise via alternative splicing of the carboxyl-terminal tail. Unlike MCP-1, CCR2 expression is relatively restricted to certain types of cells. CCR2A is the major isoform expressed by mononuclear cells and vascular smooth muscle cells, while monocytes and activated NK cells, express predominantly the CCR2B isoform. It is possible that CCR2A and CCR2B may activate different signaling pathway and exert different actions. In addition, there may be another alternative MCP-1 receptor which may be important in mediating some of the effects of MCP-1 in atherosclerotic arteries and in other inflammatory processes.[2] Both MCP-1 and its receptor CCR2 have been demonstrated to be induced and involved in a variety of diseases. Analysis of the signal transduction pathways triggered by MCP-1 has revealed that it induces a pertussis toxin (PTX)-sensitive rise of intracellular calcium, inhibition of adenyl cyclase, phospholipase C activation, activation of extracellular signal-related kinases (ERKs) and the 2 stress-activated protein kinases (SAPKs), JNK1 and P38, stimulation of 2 separate PI 3-kinase isoforms, namely p85/p110 PI3-kinase (PI3-K) and PI3K-C2alpha. Recently, analysis of signaling events in human monocytic cells has shown that MCP-1 triggers tyrosine phosphorylation and activation of the JAK2/STAT3 pathway in a PTX-independent manner.[3]

### Reference


1. Gerard, C. & and Rollins, B.J.: Nature Immunol. 2:108-115, 2001
2. Schecter, S.D. et al: J. Leuk. Biol. 75:1079-1085, 2004
3. Cambien, B. et al: Blood 97:359-66, 2001


## ELISA OVERVIEW

Cell Applications ELISA Kits are based on standard sandwich enzyme-linked immunosorbent assay technology. Freshly prepared standards, samples, and solutions are recommended for best results.

1. Prepare test samples.
2. Prepare a protein standard of the target protein.
3. Add test samples and standards to the pre-coated 96-well plate. Do not wash.
4. Add biotinylated detection antibodies. Wash.
5. Add Avidin-Biotin-Peroxidase Complex (ABC) Solution. Wash.
6. Add Tetramethyllbenzidine (TMB) Color Developing Agent, containing HRP substrate.
7. Add TMB Stop Solution
8. Subject the plate to analysis.

## NOTES:

- Before using the kit, quick spin tubes to bring down all solution to the bottom of tube.
- Duplicate assay wells are recommended for both standard and sample testing.
- Do not let the 96-well plate dry, this will lead to inactivation of plate components.
- When diluting samples and reagents, ensure that they are mixed completely and evenly.
- Pre-warm diluted ABC and TMB solutions at 37°C for 30 min before use to avoid variable temperature effects.
- For washes, use TBS or PBS. Do not touch well walls.
- A protein standard is included in the kit. A protein standard detection curve should be generated with each experiment, no more than 2 hours prior to the experiment.
- The user will determine sample dilution fold by estimation of target protein amount in samples.

## ELISA PROTOCOL

### Preparation of Test Samples

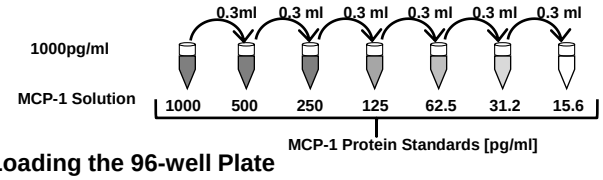
1. Process Test Samples in the following manner:
   - **Cell culture supernate, tissue lysate or body fluids**: Remove particulates by centrifugation.
   - **Serum**: Allow the serum to clot in a serum separator tube (about 2 hours) at room temperature. Centrifuge at approximately 1000 x g for 10 min. Analyze the serum immediately or aliquot and store frozen at -20° C.
   - **Plasma:** Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 10 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20° C. Heparin and citrate are not recommended as the anticoagulant..
2. Estimate the concentration of the target protein in the sample and select a proper dilution factor such that the diluted target protein concentration falls within the 15.6-1000 pg/ml standard curve range. Depending on the sample, several trial dilutions may be necessary. Dilute the sample using the provided diluent buffer, mixing well. Suggested working dilutions of samples are as follows:

| Target Protein Concentration Range | Sample Working Dilution | Sample Vol. | Diluent Buffer Vol. |
|---|---|---|---|
| 10-100 ng/ml | 1:100 | 1 µl | 99 µl |
| 1-10 ng/ml | 1:10 | 10 µl | 90 µl |
| 15.6-1000 pg/ml | 1:2 | 50 µl | 50 µl |
| ≤15.6 pg/ml | n/a | 100µl | n/a |

3. If samples will be assayed within 24 hours, store at 2-8° C. For long-term storage, aliquot and freeze samples at -20° C. Avoid repeated freeze-thaw cycles.

### Preparation of Standard Solutions (15.6-1000 pg/ml)

4. Reconstitute the Lypophilized Recombinant Protein to make a 10.000 pg/ml human MCP-1 solution. Add 1 ml Sample Diluent Buffer to a tube of lypophilized protein, keep the tube at room temperature for 10 min. Mix thoroughly.
5. Add 0.1 ml of the mixed 10,000 pg/ml MCP-1 solution to the eppendorf tube containing 0.9 ml diluent buffer and mix to make a 1000 pg/ml MCP-1 solution.
6. Label 6 eppendorf tubes with the human MCP-1 protein concentrations to be prepared by serial dilution :500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml
7. Aliquot 0.3 ml of the Sample Diluent Buffer to the labeled tubes.
8. Serially dilute the protein standards into their respectively labeled tubes. Transfer 0.3 ml from the 1000pg/ml MCP-1 Solution to the 500pg/ml eppendorf tube and mix thoroughly. Transfer 0.3 ml of the 500 pg/ml solution to the 250pg/ml tube and mix thoroughly, Transfer 0.3 ml of the 250pg/ml solution to the 125pg/ml tube and mix, and so on to make the 62.5, 31.3 and 15.6 pg/ml solutions.
9. Store at 4° C until use.




### Loading the 96-well Plate

10. Aliquot 0.1 ml of the sample diluent buffer into a control well to serve as the Blank. This will yield the O.D.450(Blank) reading.
11. Aliquot 0.1 ml of the standard solutions of the **Preparation of Standard Solutions** (15.6-1000pg/ml) into empty wells of the precoated 96-well plate. Duplicate measurements of standards are recommended.
12. Aliquot 0.1 ml of each properly diluted test sample to empty wells prepared in **Step 2**. Duplicate measurements of each test sample are recommended.
13. Cover the 96-well plate and incubate at 37° C for 90 min.
14. During the **Step 13** incubation period, prepare a stock of Biotinylated 1:100 Antibody Working Solution. Count the number of reactions and multiply by 0.1 ml/well for the Working Solution total volume (preparation of 1-2 reactions in excess of the number of wells is recommended). Dilute the Biotinylated Antibody to 1:100 in Antibody Diluent Buffer and mix thoroughly. Use the working solution within 2 hours.
15. Upon completion of the 90 min incubation of **Step 13**, remove the cover of the 96 well plate and discard plate well contents. Blot the plate onto paper towels or other absorbent material. DO NOT let the wells completely dry at any time.
16. Add 0.1 ml of the Biotinylated 1:100 Antibody Working Solution (prepared in **Step 14**) to each well and incubate the plate at 37° C for 60 min.
17. During the incubation period of **Step 16**, prepare a stock of ABC Working Solution. Count the number of reactions and multiply by 0.1 ml/well for the Working Solution total volume (preparation of 1-2 reactions in excess of the number of wells is recommended). Dilute the ABC Stock Solution to 1:100 in ABC Diluent Buffer and mix thoroughly. Pre-warm the ABC working solution at 37° C for 30 min before use. Use the working solution within 1 hour.
18. Upon completion of the 60 min incubation of **Step 16**, wash the plate 3 times with 0.3 ml TBS or PBS. For each wash, leave washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
19. Add 0.1 ml of prepared ABC Working Solution (prepared in **Step 17**) to each well and incubate the plate at 37° C for 30 min.
20. Add 90 µl of the pre-warmed TMB Color Developing Agent into each well and incubate at 37° C for 14-18 min (shades of blue can be seen in the wells with the four most concentrated Protein Standard Solutions; the other control wells should show no obvious color).
21. During the incubation period of **Step 19**, pre-warm TMB Color Developing Agent at 37° C for 30 min before use.
22. Upon completion of the 30 min incubation of **Step 19**, wash the plate 5 times with 0.3 ml TBS or PBS. For each wash, leave the washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
23. Add 0.1 ml of the TMB Stop Solution to each well. The acidic stop solution will change the mixture color to yellow. The yellow intensity is proportional to the amount of target protein captured by the plate.
24. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution. These readings are the O.D.450(Reading).

### Calculating Protein Concentration

- For all wells, determine O.D.450(Relative):
  O.D.450(Relative) = O.D.450(Reading) – O.D.450(Blank)
- Plot the standard curve: plot O.D.450(Relative) of each standard solution (Y) vs. the respective concentration of the standard solution (X). See **Figure 1** for a typical standard curve.
- The human MCP-1 concentration of the samples can be interpolated from the standard curve. Multiply the interpolated concentration by the dilution factor to obtain the target protein concentration in the sample.