



Product Information Sheet

Human MCP-1 ELISA Kit

Catalog No. EK0441
Size 96T
Range 15.6pg/ml-1000pg/ml
Sensitivity < 1pg/ml
Specificity
 Cross-reactivates with human Eotaxin, MCP-3 <1%.

Storage
 Store at 4 °C for frequent use, at -20 °C for infrequent use.
 Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Expiration
 Four months at 4 °C and eight months at -20 °C.

Application
 For quantitative detection of human MCP-1 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

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Principle

Human MCP-1 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human MCP-1 specific polyclonal antibodies were precoated onto 96-well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human MCP-1 amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant human MCP-1 standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- human MCP-1 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human MCP-1 antibody : 130µl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC) : 130µl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

Material Required But Not Provided

1. Microplate reader in standard size and Automated plate washer.
2. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
3. Clean tubes and Eppendorf tubes.
4. Washing buffer (neutral PBS or TBS).

Preparation of 0.01M **TBS**: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H₂O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M **PBS**: Add 8.5g sodium chloride, 1.4g Na₂HPO₄ and 0.2g NaH₂PO₄ to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

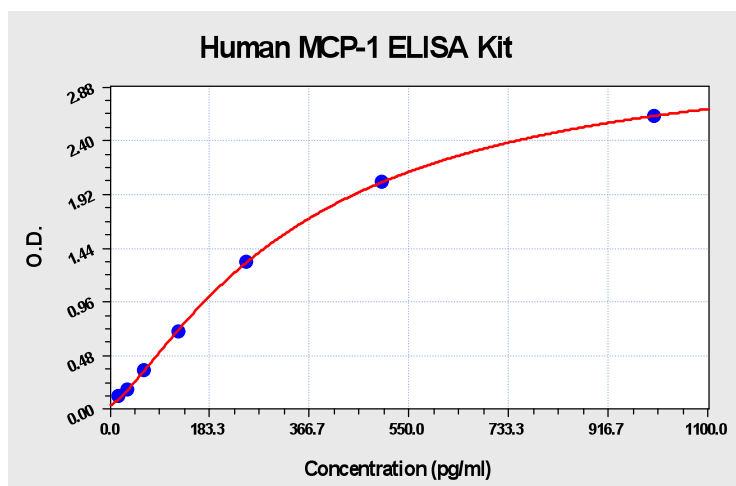
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Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

Human MCP-1 ELISA Kit-1X96 Well Plate Image



Background

Monocyte chemoattractant protein-1 (MCP-1), a member of the chemokine (chemotactic cytokine) family, is a potent monocyte agonist that is upregulated by oxidized lipids.¹ MCP-1 is also known as CCL2, SCYA2, MCAF. MCAF is a member of family of factors involved in immune and inflammatory responses. The amino acid sequence deduced from the nucleotide sequence reveals the primary structure of the MCAF precursor to be composed of a putative signal peptide sequence of 23 amino acid residues and a mature MCAF sequence of 76 amino acid residues.² MCP-1 plays a unique and crucial role in the initiation of atherosclerosis and may provide a new therapeutic target in this disorder.³ Human MCP-1 is a 8.7KDa non-glycoprotein, consisting of 99 amino acids in precursor form and 76 amino acids in mature form.

Reference

1. Gosling, J.; Slaymaker, S.; Gu, L.; Tseng, S.; Zlot, C. H.; Young, S. G.; Rollins, B. J.; Charo, I. F. MCP-1 deficiency reduces susceptibility to atherosclerosis in mice that overexpress human apolipoprotein B. *J. Clin. Invest.* 103: 773-778, 1999.
2. Furutani, Y.; Nomura, H.; Notake, M.; Oyamada, Y.; Fukui, T.; Yamada, M.; Larsen, C. G.; Oppenheim, J. J.; Matsushima, K. Cloning and sequencing of the cDNA for human monocyte chemotactic and activating factor (MCAF). *Biochem. Biophys. Res. Commun.* 159: 249-255, 1989.
3. Gu, L.; Okaka, Y.; Clinton, S. K.; Gerard, C.; Sukhova, G. K.; Libby, P.; Rollins, B. J. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Molec. Cell* 2: 275-281, 1998.

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