



Product Information Sheet

Mouse ICAM-1 ELISA Kit

Catalog No. EK0371
Size 96T
Range 156pg/ml-10,000pg/ml
Sensitivity < 10pg/ml

Specificity

No detectable cross-reactivity with any other cytokine.

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 mouse ICAM-1 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle

Antagene's mouse ICAM-1 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Mouse ICAM-1 specific-specific monoclonal antibodies were precoated onto 96-well plates. The mouse 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 mouse ICAM-1 amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant mouse ICAM-1 standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- mouse ICAM-1 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- mouse ICAM-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.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. 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

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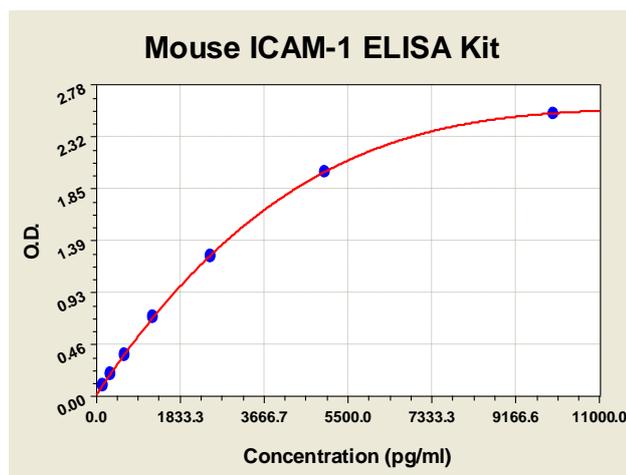
and 0.2g NaH₂PO₄ to 1000ml
distilled water and adjust pH to

7.2-7.6. Finally, adjust the total volume to 1L.

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.

Mouse ICAM-1 ELISA Kit-1X96 Well Plate Image



Background

Intercellular adhesion molecule-1 (ICAM-1) is an integral membrane protein, a member of the immunoglobulin superfamily, and a ligand for lymphocyte function-associated (LFA) antigens, a beta 2 leukocyte integrin.¹ The normal function of human intercellular adhesion molecule-1 (ICAM-1) is to provide adhesion between endothelial cells and leukocytes after injury or stress. ICAM-1 binds to leukocyte function-associated antigen (LFA-1) or macrophage-1 antigen (Mac-1).² ICAM-1 is found on leukocytes, fibroblasts, epithelial cells and endothelial cells and its expression is regulated by inflammatory cytokines.³ ICAM-1 has a tissue distribution similar to that of the major histocompatibility complex class II antigens and is likely to play a role in inflammatory responses.¹ The standard product used in this kit is produced by recombinant gene expression, which is a dimeric protein linked with disulfide bonds. The single chain is Gln28-Asn 485. It has 457 amino acids with the molecular mass of 76.8kDa. As a result of glycosylation, the molecular mass of 110-130KDa is revealed by SDS-PAGE.

Reference

1. Ballantyne, C. M.; Kozak, C. A.; O'Brien, W. E.; Beaudet, A. L. Assignment of the gene for intercellular adhesion molecule-1 (Icam-1) to proximal mouse chromosome 9. *Genomics* 9: 547-550, 1991.
2. Bella, J.; Kolatkar, P. R.; Marlor, C. W.; Greve, J. M.; Rossmann, M. G. The structure of the two amino-terminal domains of human ICAM-1 suggests how it functions as a rhinovirus receptor and as an LFA-1 integrin ligand. *Proc. Nat. Acad. Sci.* 95: 4140-4145, 1998.
3. Simmons, D.; Makgoba, M. W.; Seed, B. ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. *Nature* 331: 624-627, 1988.

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