



Product Information Sheet

Mouse VCAM-1 ELISA Kit

Catalog No.	EK0538
Size	96T
Range	156pg/ml-10,000pg/ml
Sensitivity	< 5 pg/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 VCAM-1 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle

Mouse VCAM-1 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Mouse VCAM-1 specific polyclonal 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 VCAM-1 amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant mouse VCAM-1 standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- mouse VCAM-1 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- mouse VCAM-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 if there is a large amount of samples for 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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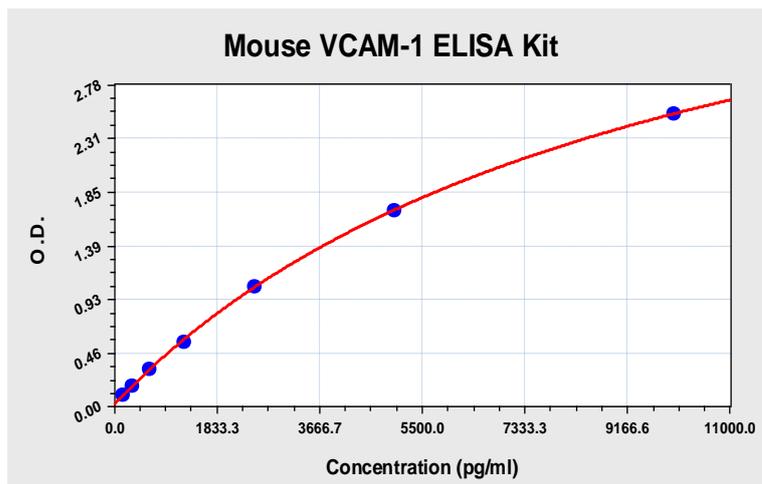
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Notice for Application of Kit

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.

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



Background

Vascular cell adhesion molecule 1 (VCAM-1) is a cell surface glycoprotein adhesive for certain blood leukocytes and tumor cells, which is expressed by activated endothelium in a variety of pathologic conditions including atherosclerosis.¹ Increased expression of VCAM1 is associated with a variety of chronic inflammatory conditions, making its expression and function a target for therapeutic intervention.² Integrin alpha4beta1 (VLA-4) and VCAM-1 facilitate a critical cell-cell adhesion event required for survival of endothelial and mural cells during vascularization.³ The VCAM1 gene is assigned to the 1p31-32 region of chromosome 1 based on the analysis of human-mouse hybrid cell lines and in situ hybridization.¹ The standard product used in this kit is recombinant mouse VCAM-1, a dimeric protein linking with a disulfide bond. It consists of Phe25-Glu698 amino acid sequence of a single chain with the molecular mass of 101.9KDa. As a result of glycosylation, the molecular mass of 120KDa is revealed by SDS-PAGE.

Reference

1. Cybulsky, M. I.; Fries, J. W. U.; Williams, A. J.; Sultan, P.; Eddy, R.; Byers, M.; Shows, T.; Gimbrone, M. A., Jr.; Collins, T. Gene structure, chromosomal location, and basis for alternative mRNA splicing of the human VCAM1 gene. *Proc. Nat. Acad. Sci.* 88: 7859-7863, 1991.
2. Besemer, J.; Harant, H.; Wang, S.; Oberhauser, B.; Marquardt, K.; Foster, C. A.; Schreiner, E. P.; de Vries, J. E.; Dascher-Nadel, C.; Lindley, I. J. D. Selective inhibition of cotranslational translocation of vascular cell adhesion molecule 1. (Letter) *Nature* 436: 290-293, 2005.
3. Garmy-Susini, B.; Jin, H.; Zhu, Y.; Sung, R.-J.; Hwang, R.; Varner, J. Integrin alpha-4-beta-1--VCAM-1--mediated adhesion between endothelial and mural cells is required for blood vessel maturation. *J. Clin. Invest.* 115: 1542-1551, 2005.

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