



Product Information Sheet

Human soluble CD40L ELISA Kit

Catalog No. EK0573
Size 96T
Range 62.5pg/ml-4000pg/ml
Sensitivity < 6pg/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 human CD40L in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle

Human CD40L ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human CD40L 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 CD40L amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant human CD40L standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- human CD40L antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human CD40L 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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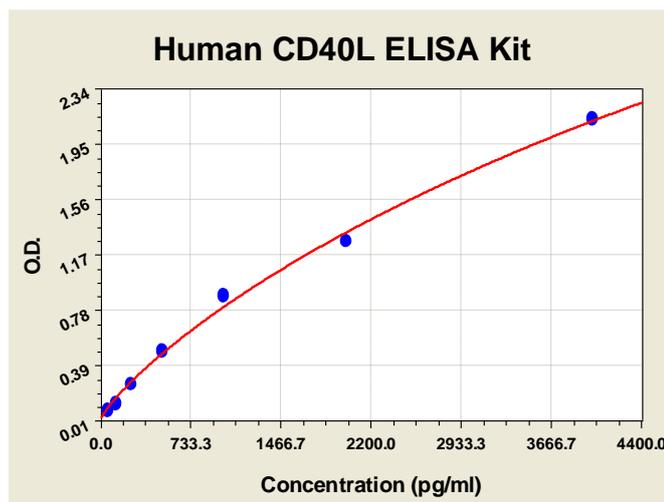
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Notice for Application of Kit

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.

Human CD40L ELISA Kit-1X96 Well Plate Image



Background

CD40 ligand (CD40L) is a type II membrane protein of 261 amino acids on activated T cells that induces B cell proliferation and immunoglobulin secretion. It has homology with tumour necrosis factor- α and - β , and has important functions in B-cell activation and differentiation. Human CD40L with 5 exons, is mapped to the proximal region of the mouse X chromosome on Xq26.3-27.1, and can be detected on T cells but is absent from B cells and monocytes. Since CD40L is expressed on platelets and released from them on activation, its predictive value as a marker for clinical outcome and the therapeutic effect of inhibition of glycoprotein IIb /IIIa receptor in patients with acute coronary syndromes was investigated. The soluble CD40L may be involved in the process of restenosis and that it exerts its effect by triggering a complex group of inflammatory reactions on endothelial and mononuclear cells. CD40L plays a central role in the pathophysiology of acute coronary syndromes, and has a role in the pathogenesis of coronary artery lesions.

Reference

1. Allen, R.C.; Armitage, R.J.; Conley, M.E.; Rosenblatt, H.; Jenkins, N.A.; Copeland, N.G.; Bedell, M.A.; Edelhoff, S.; Disteche, C. M.; Simoneaux, D.K.; Fanslow, W.C.; Belmont, J.; Spriggs, M.K.: CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* 259: 990-993, 1993.
2. Cipollone, F.; Ferri, C.; Desideri, G.; Paloscia, L.; Materazzo, G.; Mascellanti, M.; Fazio, M.; Iezzi, A.; Cuccurullo, C.; Pini, B.; Bucchi, M.; Santucci, A.; Cuccurullo, F.; Mezzetti, A.: Preprocedural level of soluble CD40L is predictive of enhanced inflammatory response and restenosis after coronary angioplasty. *Circulation* 108: 2776-2782, 2003.