

Product Information Sheet

Human VEGF-C ELISA Kit

Catalog No. EK0588
Size 96T
Range 31.2pg/ml-2000pg/ml
(human sera, body fluids)
Sensitivity < 2pg/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 VEGF-C in sera, serum, body fluids, tissue lysates or cell culture supernates.

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Principle

Human VEGF-C ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human VEGF-C specific-specific polyclonal antibodies were precoated onto 96-well plates. The human specific detection monoclonal 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 VEGF-C amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant human VEGF-C standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- human VEGF-C antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human VEGF-C 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.

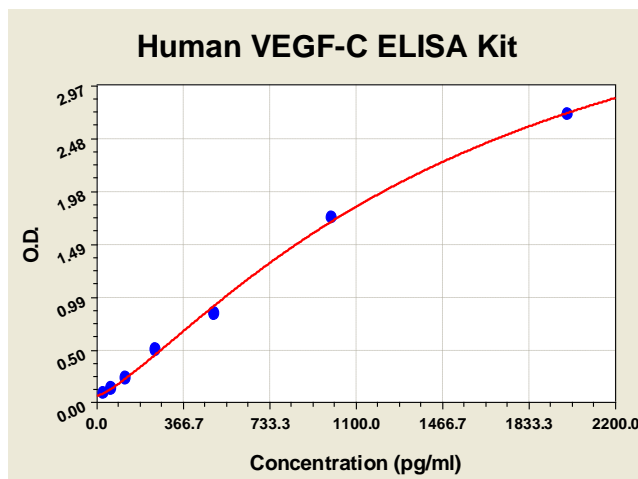
Preparation of 0.01 M **PBS**: Add 8.5g sodium chloride, 1.4g Na₂HPO₄ and 0.2g NaH₂PO₄ to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

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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 VEGF-C ELISA Kit-1X96 Well Plate Image



Background

Vascular endothelial growth factor C is a VEGF. The human gene encoding it is VEGFC. The protein encoded by this gene is a member of the platelet-derived growth factor/vascular endothelial growth factor (PDGF/VEGF) family, is active in angiogenesis, lymphangiogenesis and endothelial cell growth and survival, and can also affect the permeability of blood vessels. This secreted protein undergoes a complex proteolytic maturation, generating multiple processed forms which bind and activate VEGFR-3 receptors. Only the fully processed form can bind and activate VEGFR-2 receptors. This protein is structurally and functionally similar to vascular endothelial growth factor D (VEGF-D). The C terminus of VEGFC has cysteine-rich repeat units characteristic of the Balbiani ring 3 protein (BR3P) of the midge *Chironomus tentans*.^{1,2} The standard product used in this kit is recombinant human VEGF-C, consisting of 135 amino acids with the molecular mass of 23Kda after glycosylation.

Reference

1. Joukov, V.; Pajusola, K.; Kaipainen, A.; Chilov, D.; Lantinen, I.; Kukk, E.; Saksela, O.; Kalkkinen, N.; Alitalo, K. : A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. *EMBO J.* 15: 290-298, 1996.
2. Lee, J.; Gray, A.; Yuan, J.; Luoh, S.-M.; Avraham, H.; Wood, W. I. : Vascular endothelial growth factor-related protein: a ligand and specific activator of the tyrosine kinase receptor Flt4. *Proc. Nat. Acad. Sci.* 93: 1988-1992, 1996.