



Product Information Sheet

Human Fractalkine/CX3CL1 ELISA Kit

Catalog No. EK0356

Size 96T

Range 156pg/ml-10,000pg/ml

Sensitivity < 50pg/ml

Specificity
Detectable cross-reactivity with Eotaxin, MCP-1, MCP-2, 6Ckine, MARC < 0.2%.

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 Fractalkine in sera, plasma, body fluids, tissue lysates or cell culture supernates.

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Principle

Antagene's human Fractalkine ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human Fractalkine specific-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 Fractalkine amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant human Fractalkine standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- human Fractalkine antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human Fractalkine 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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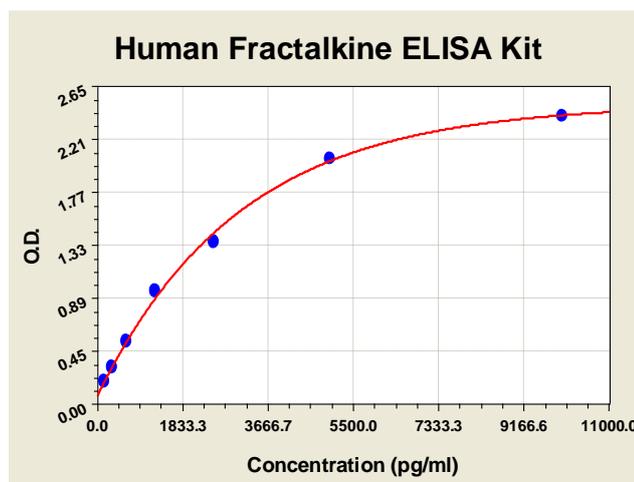
and 0.2g NaH₂PO₄ to 1000ml distilled water and adjust pH to

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.

Human Fractalkine ELISA Kit-1X96 Well Plate Image



Background

Chemokine (C-X₃-C motif) ligand 1 (CX₃CL1) is a large cytokine protein of 373 amino acids, it contains multiple domains and is the only known member of the CX₃C chemokine family. It is also commonly known under the names fractalkine (in humans) and neurotactin (in mice).^{1,2} CX₃CL1 is produced as a long protein (with 373-amino acid in humans) with an extended mucin-like stalk and a chemokine domain on top. The mucin-like stalk permits it to bind to the surface of certain cells. However a soluble (90 kD) version of this chemokine has also been observed. Soluble CX₃CL1 potently chemoattracts T cells and monocytes, while the cell-bound chemokine promotes strong adhesion of leukocytes to activated endothelial cells, where it is primarily expressed.² CX₃CL1 elicits its adhesive and migratory functions by interacting with the chemokine receptor CX₃CR1.³ Its gene is located on human chromosome 16 along with some CC chemokines known as CCL17 and CCL22.^{2,4} It can act as a mediator of smooth muscle cell migration in human atherosclerosis, rather than mediate inflammatory cell recruitment.⁵ The standard product used in this kit is recombinant human CX₃CL1, consisting of 76 amino acids with the molecular mass of 8.5kDa.

Reference

1. Pan et al. Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. *Nature* 387: 611-617, 1997.
2. Bazan et al. A new class of membrane-bound chemokine with a CX₃C motif. *Nature* 385: 640-644, 1997.
3. Imai et al. Identification and molecular characterization of fractalkine receptor CX₃CR1, which mediates both leukocyte migration and adhesion. *Cell* 91: 521-530, 1997.
4. Nomiya et al. Human chemokines fractalkine (SCYD1), MDC (SCYA22) and TARC (SCYA17) are clustered on chromosome 16q13. *Cytogenet. Cell Genet.* 81: 10-11, 1998.
5. Lucas, A. D.; Bursill, C.; Guzik, T. J.; Sadowski, J.; Channon, K. M.; Greaves, D. R. : Smooth muscle cells in human atherosclerotic plaques express the fractalkine receptor CX₃CR1 and undergo chemotaxis to the CX₃C chemokine fractalkine (CX₃CL1). *Circulation* 108: 2498-2504, 2003.

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