Product Information Sheet

Rat Fibronectin ELISA Kit

Catalog No. EK0350

Size 96T

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

Sensitivity < 15pg/ml

Specificity

No detectable cross-reactivity with any other cytokine.

Storage

Store at 4℃ for frequent use, at -20℃ for infrequent use.

Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Expiration

Four months at 4℃ and eight months at -20℃.

Application

For quantitative detection of rat Fibronectin in sera, plasma, body fluids, tissue lysates or cell culture supernates.

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Principle

Rat Fibronectin ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Rat Fibronectin specific-specific polyclonal antibodies were precoated onto 96-well plates. The rat 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 termination solution. The density of yellow is proportional to the rat Fibronectin amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant rat Fibronectin standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- rat Fibronectin antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- rat Fibronectin 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 H_2O 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 Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

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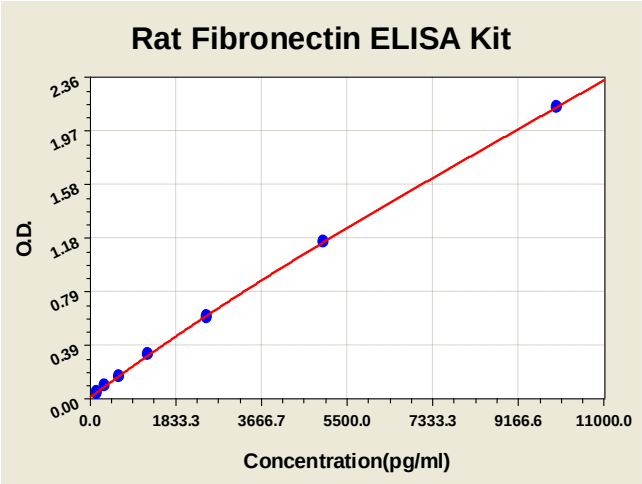
Notice for Application of Kit

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℃ for 30 min before using.

Rat Fibronectin ELISA Kit-1X96 Well Plate Image



Background

Fibronectin (FN) also known as LETS, is identified on the surfFN of fibroblasts by labeling with radioactive compounds or specific antibodies. Fibronectin is a 430,000-dalton dimeric glycoprotein that exists in 2 forms, termed cellular and plasma fibronectin. Cellular and plasma fibronectins are heterodimers consisting of similar but not identical polypeptides.[1] These two forms of FN differ in biologic activity. Fibronectins bind cell surfFNs and various compounds including collagen, fibrin, heparin, DNA, and actin. Because fibronectin stimulates endocytosis in several systems and promotes the clearance of particulate material from the circulation, it could function in the clearance of C1q-coated material such as immune complexes or cellular debris.[2] Fibronectins are involved in cell adhesion, cell motility, opsonization, would healing, and maintenance of cell shape. LETS, encoded on chromosome 8, is responsible for the LETS protein expression in rats. Because LETS has been implicated in tumorigenicity and cellular transformation, it is of interest that rearrangement or modifications in the number of chromosome 8 have been associated with certain forms of cancer.[3] The standard product used in this kit is isolated from rat plasma with the molecular mass of 200-250KDa.

Reference

1. Kornblihtt, A. R.; Umezawa, K.; Vibe-Pedersen, K.; Baralle, F. E. Primary structure of rat fibronectin: differential splicing may generate at least 10 polypeptides from a single gene. *EMBO J.* 4: 1755-1759, 1985.
2. Bing, D. H.; Almeda, S.; Isliker, H.; Lahav, J.; Hynes, R. O. Fibronectin binds to the C1q component of complement. *Proc. Nat. Acad. Sci.* 79: 4198-4201, 1982.
3. Owerbach, D.; Doyle, D.; Shows, T. B. Genetics of the large, external, transformation-sensitive (LETS) protein: assignment of a gene coding for expression of LETS to rat chromosome 8. *Proc. Nat. Acad. Sci.* 75: 5640-5644, 1978.