



Product Information Sheet

Rat PDGF-AB ELISA Kit

Catalog No.	EK0485
Size	96T
Range	31.2pg/ml-2000pg/ml
Sensitivity	< 3 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 rat PDGF-AB in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle

Rat PDGF-AB ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Rat PDGF-AB 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 stop solution. The density of yellow is proportional to the rat PDGF-AB amount of sample captured in plate.

Kit Components

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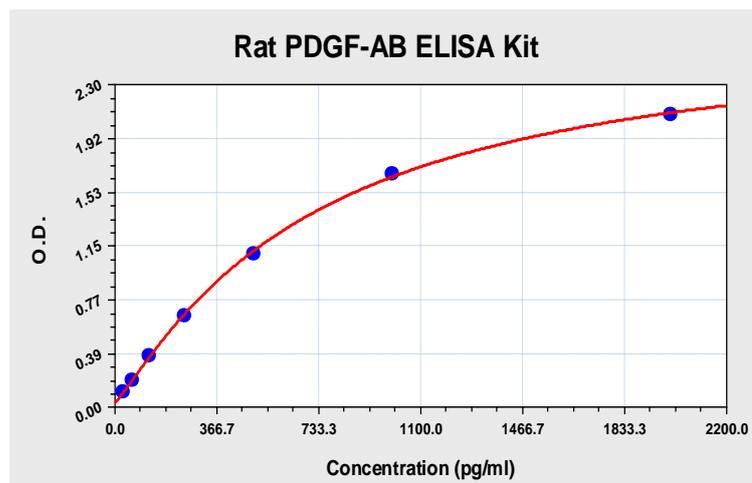
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Notice for Application of Kit

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.

Rat PDGF-AB ELISA Kit-1X96 Well Plate Image



Background

The platelet-derived growth factor (PDGF) is a mitogen derived from human platelets consisting of two related polypeptides termed A and B chains.¹ The genes for PDGF A chain, B chain/c-sis, and the PDGF receptor are expressed in human malignant glioma cell lines.² Normal human endothelial cells in culture express the B chain of PDGF, and that endothelial-derived PDGF B chain is synthesized as a predicted precursor polypeptide of Mr 27,281.³ The entire B chain of PDGF is highly (96%) homologous to a portion of p28sis, the transforming protein of simian sarcoma virus (SSV). It has been suggested that p28sis exerts its transforming potential by mimicking the growth promoting activity of PDGF and stimulating the cell in an autocrine manner.¹ PDGF A-chain precursor polypeptide is assigned to the proximal long arm of chromosome 7, band q11.23.⁴ The human homolog (PDGF B-chain/c-sis) of the transforming gene of simian sarcoma virus is assigned to chromosome 22.⁵ The standard product used in this kit is recombinant rat PDGF-AB(A chain Ser87-Arg196; B chain Ser74-Thr182) with the molecular mass of 24.8KDa.

Reference

1. Kelly, J. D.; Raines, E. W.; Ross, R.; Murray, M. J. The B chain of PDGF alone is sufficient for mitogenesis. *EMBO J.* 4: 3399-3405, 1985.
2. Hermansson, M.; Nister, M.; Betsholtz, C.; Heldin, C.-H.; Westermarck, B.; Funai, K. Endothelial cell hyperplasia in human glioblastoma: coexpression of mRNA for platelet-derived growth factor (PDGF) B chain and PDGF receptor suggests autocrine growth stimulation. *Proc. Nat. Acad. Sci.* 85: 7748-7752, 1988.
3. Collins, T.; Ginsburg, D.; Boss, J. M.; Orkin, S. H.; Pober, J. S. Cultured human endothelial cells express platelet-derived growth factor B chain: cDNA cloning and structural analysis. *Nature* 316: 748-750, 1985.
4. Stenman, G.; Rorsman, F.; Betsholtz, C. Sublocalization of the human PDGF A-chain gene to chromosome 7, band q11.23, by in situ hybridization. *Exp. Cell Res.* 178: 180-184, 1988.
5. Dalla-Favera, R.; Gallo, R. C.; Giallongo, A.; Croce, C. Chromosomal localization of the human homolog (c-sis) of the simian sarcoma virus onc gene. *Science* 218: 686-688, 1982.

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