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Human PDGF-AB ELISA Kit

Catalog No. EA100339
Size 96T(8×12 divisible strips)

For quantitative detection of human PDGF-AB in cell culture supernates, serum and plasma(heparin, EDTA).

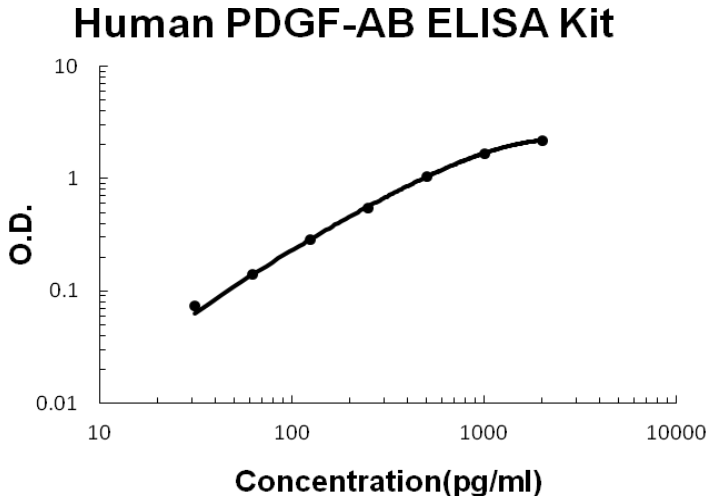
Typical Data Obtained from Human PDGF-AB

(TMB reaction incubate at 37°C for 19 min)

Concentration(pg/ml)	0.0	31.2	62.5	125	250	500	1000	2000
O.D	0.001	0.074	0.139	0.284	0.551	1.053	1.683	2.194

Typical Human PDGF-AB ELISA Kit Standard Curve

This standard curve was generated at OriGene for demonstration purpose only. A standard curve must be run with each assay.



Range	31.2pg/ml-2000pg/ml
Sensitivity	< 2pg/ml
Specificity	Natural and recombinant human PDGF-AB
Cross-reactivity	No detectable cross-reactivity with other relevant proteins

Storage

Store at 4°C for 6 months, at -20°C for12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	237	532	974	254	683	1205
Standard deviation	11.6	27.7	62.4	13.97	45.8	95.2
CV(%)	4.9	5.2	6.3	5.5	6.7	7.9

Principle

OriGene's human PDGF-AB ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for PDGF-AB has been precoated onto 96-well plates. Standards(E.coli, (S87-T211)+(S82-T190)) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for PDGF-AB is added 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 PDGF-AB amount of sample captured in plate.

Kit Components

Description	Quantity
96-well plate precoated with anti- human PDGF-AB antibody	1
Lyophilized recombinant human PDGF-AB standard	10ng/tube×2
Biotinylated anti- human PDGF-AB antibody	130µl(dilution 1:100)
Avidin-Biotin-Peroxidase Complex (ABC)	130µl(dilution 1:100)
Sample diluent buffer	30 ml
Antibody diluent buffer	12ml
ABC diluent buffer	12ml
TMB color developing agent	10ml
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.

Notice for Application of Kit

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Duplicate well assay is recommended for both standard and sample testing.
5. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
6. Don't reuse tips and tubes to avoid cross contamination.
7. Avoid using the reagents from different batches together.
8. 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.

Preparation

1. **Sample Preparation and Storage**

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Cell culture supernate**: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C.
- **Serum**: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C
- **Plasma**: Collect plasma using **heparin** or **EDTA** as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. For eliminating platelet, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20°C. Citrate is not recommended as the anticoagulant..

2. **Sample Dilution Guideline**

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluents buffer.**

- **High target protein concentration (20-200ng/ml).** The working dilution is 1:100. i.e. Add 1μl sample into 99 μl sample diluent buffer.
- **Medium target protein concentration (2-20ng/ml).** The working dilution is 1:10. i.e. Add 10μl sample into 90 μl sample diluent buffer.
- **Low target protein concentration (31.2-2000pg/ml).** The working dilution is 1:2. i.e. Add 50μl sample to 50 μl sample diluent buffer.
- **Very Low target protein concentration ≤ 31.2pg/ml).** No dilution necessary, or the working dilution is 1:2.

3. **Reagent Preparation and Storage**

A. Reconstitution of the human PDGF-AB standard: PDGF-AB standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of PDGF-AB standard (10ng per tube) are included in each kit. Use one tube for each experiment.

   a. 10,000pg/ml of human PDGF-AB standard solution: Add 1ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.

b. 2000pg/ml of human PDGF-AB standard solution: Add 0.2ml of the above 10ng/ml PDGF-AB standard solution into 0.8 ml sample diluent buffer and mix thoroughly.

c. 1000pg/ml→31.2pg/ml of human PDGF-AB standard solutions: Label 6 Eppendorf tubes with 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml respectively. Aliquot 0.3ml of the sample diluent buffer into each tube. Add 0.3ml of the above 2000pg/ml PDGF-AB standard solution into 1st tube and mix. Transfer 0.3ml from 1st tube to 2nd tube and mix. Transfer 0.3ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-human PDGF-AB antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2ml more than total volume)

b. Biotinylated anti-human PDGF-AB antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1µl Biotinylated anti-human PDGF-AB antibody to 99µl antibody diluent buffer.)

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1µl ABC to 99µl ABC diluent buffer.)

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard PDGF-AB detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of PDGF-AB amount in samples.

1. Aliquot 0.1ml per well of the 2000pg/ml,1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml human PDGF-AB standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of human cell culture supernates, serum or plasma(heparin, EDTA) to each empty well. **See "Sample Dilution Guideline" above for details.** It is recommended that each human PDGF-AB standard solution and each sample be measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human PDGF-AB antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (**Plate Washing Method:** Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.

7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 15-20 min (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human PDGF-AB standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative $O.D._{.450}$) = (the $O.D._{.450}$ of each well) – (the $O.D._{.450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{.450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human PDGF-AB concentration of the samples can be interpolated from the standard curve. **Note:** if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 15-20 min.
5. Add TMB stop solution and read.

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 human PDGF-AB with the molecular mass of 27KDa.

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.; Westermark, B.; Funa, 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.