PDGF-AB ELISA Kit (Human) (OKBB00239)

Lot# KG0854

Instruction for Use

For the quantitative measurement of PDGF-AB in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

Variation between lots can occur. Refer to the manual provided with the kit.

This product is intended for research use only

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1. Background

Principle

Aviva Systems Biology PDGF-AB ELISA Kit (Human) (OKBB00239) is based on standard sandwich enzyme-linked immune-sorbent assay technology. An antibody specific for PDGF-AB has been pre-coated onto 96-wellplate (12 x 8 Well Strips). Standards (E.coli, (S87-T211)+(S82-T190)) and test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for PDGF-AB is added, incubated and followed by washing. Avidin-Biotin-Peroxidase Complex is then added, incubated and unbound complex is washed away. An enzymatic reaction is visualized through the addition of TMB substrate which is catalyzed by HRP to produce a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm and is quantitatively proportional to the amount of sample Human PDGF-AB captured in the well.

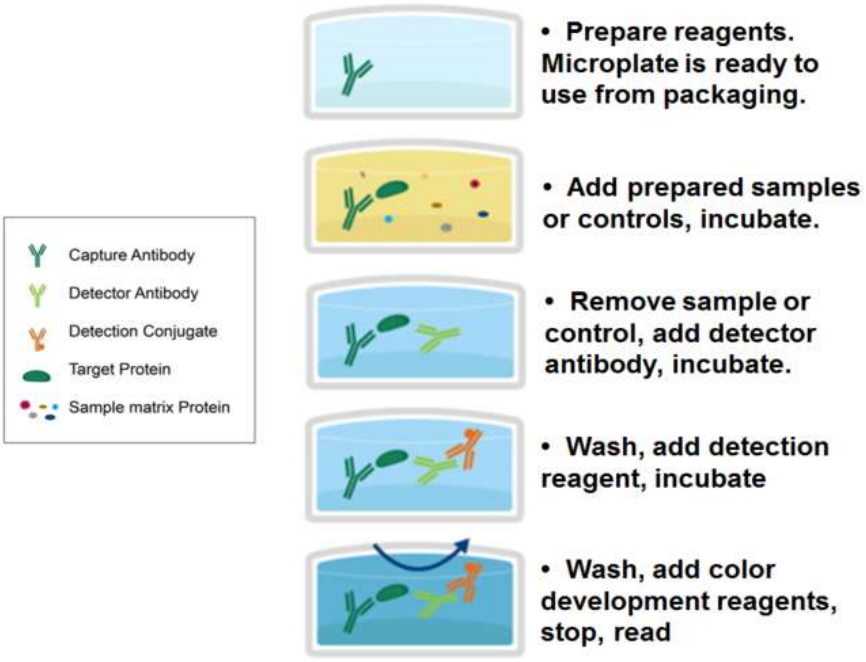
Background

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.

General Specifications

General Specifications	
Range	31.2 – 2,000 pg/mL
Sensitivity	< 2 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Natural and recombinant Human PDGF-AB UniProt ID: P04085
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Upon receipt store kit at -20°C until expiration date. Avoid multiple freeze/thaw cycles.

4. Kit Components

• The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
96-Well plate Pre-coated with Anti-Human PDGF-AB Antibody	96 Wells (12 x 8 Well Strips)	Store at -20°C until expiration date
Lyophilized Recombinant Human PDGF-AB standard	2 x 10 ng	
100X Biotinylated Anti-Human PDGF-AB Antibody	1 x 130 µL	
100X Avidin-Biotin-Peroxidase Complex (ABC)	1 x 130 µL	
Sample Diluent Buffer	1 x 30 mL	
Antibody Diluent Buffer	1 x 12 mL	
ABC Diluent Buffer	1 x 12 mL	
TMB Color Developing Agent	1 x 10 mL	
TMB Stop Solution	1 x 10 mL	
10X Wash Buffer	1 x 30 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.
• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.
• Automated plate washer (optional).
• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
• Absorbent paper or paper toweling.
• Distilled or deionized ultrapure water.

7. Technical Application Tips

• Do not mix or substitute components from other kits.
• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
• Inspect all reagents prior to use. Components should contain no particulates or cloudiness and should be colorless.
• Prior to using the kit, briefly spin component tubes to collect all reagent at the bottom.
• Replicate wells are recommended for standards and samples.
• Cover microplate while incubating to prevent evaporation.
• Do not allow the microplate wells dry at any point during the assay procedure.
• Do not reuse tips or tube to prevent cross contamination.
• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
• Completely remove of all liquids when washing to prevent cross contamination.
• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
• Equilibrate all materials to ambient room temperature prior to use (standards exception).
• For optimal results in inter- intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.
• Pipetting less than 1 µL is not recommended for optimal assay accuracy.
• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
• Samples containing precipitates or fibrin strands or which are hemolytic of lipemic might cause inaccurate results due to interfering factors.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

8.1 PDGF-AB Assay standards

8.1.1 Prepare the PDGF-AB standards no greater than 2 hours prior to performing experiment. standards should be held on ice until use in the experiment.

8.1.2 Reconstitute one of the provided 10 ng Lyophilized **Recombinant Human PDGF-AB standard. Use** one for each experiment. Prepare a stock 10,000 pg/mL **Human PDGF-AB** standard by reconstituting one tube of **Lyophilized Recombinant Human PDGF-AB standard as follows**:

8.1.2.1 Gently spin or tap the vial to collect all material at the bottom.

8.1.2.2 Add 1 mL of **Sample Diluent Buffer** to the vial.

8.1.2.3 Seal then mix gently and thoroughly.

8.1.2.4 Leave the vial to sit at ambient temperature for 10 minutes.

8.1.3 Prepare a set of seven serially diluted standards as follows:

8.1.3.1 Label tubes with numbers 1 – 8.

8.1.3.2 Add 300 µL of **Sample Diluent Buffer** to Tube #'s 1 – 7.

8.1.3.3 Prepare a **2,000 pg/mL standard #1** in by adding 200 µL of the 10,000 pg/mL reconstituted **Human PDGF-AB standard** to 800 µL of **Sample Diluent Buffer** in Tube#1. Mix gently and thoroughly.

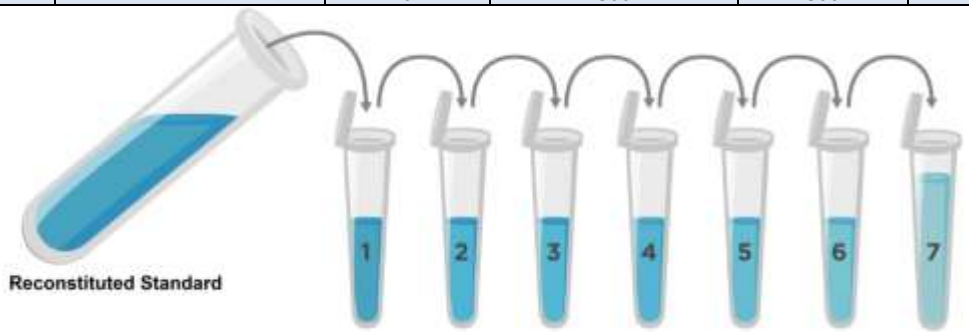
8.1.3.4 Prepare **standard #2** by adding 300 µL of **2,000 pg/mL standard#1** from Tube #1 to Tube #2. Mix gently and thoroughly.

8.1.3.5 Prepare **standard #3** by adding 300 µL of **standard #2 from** Tube #2 to Tube #3. Mix gently and thoroughly.

8.1.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.1.3.7 Tube #8 is a blank standard (only **Sample Diluent Buffer**), which should be included with every experiment.

Standard Number (Tube)	Sample To Dilute	Volume standard (µL)	Volume Sample Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
1	10,000 pg of Human PDGF-AB standard	200	800	1,000	2,000 pg/mL
2	2,000 pg/mL	300	300	600	1,000 pg/mL
3	1,000 pg/mL	300	300	600	500 pg/mL
4	500 pg/mL	300	300	600	250 pg/mL
5	250 pg/mL	300	300	600	125 pg/mL
6	125 pg/mL	300	300	600	62.5 pg/mL
7	62.5 pg/mL	300	300	600	31.2 pg/mL
8	NA	0	300	300	0.0 (Blank)



8.2 1X Biotinylated Anti-Human PDGF-AB Antibody

8.2.1 Prepare the **1X Biotinylated Anti-Human PDGF-AB Antibody immediately** prior to use by diluting the **100X Biotinylated Anti-Human PDGF-AB Antibody** 1:100 with Antibody Diluent Buffer.

8.2.2 For each well to be used in the experiment prepare 100 µL by adding 1 µL of **100X Biotinylated Anti-Human PDGF-AB Antibody to** 99 µL Antibody Diluent Buffer.

8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.3 1X Avidin-Biotin-Peroxidase Complex (ABC)

8.3.1 Prepare the **1X Avidin-Biotin-Peroxidase Complex (ABC)** immediately prior to use by diluting the **100X Avidin-Biotin-Peroxidase Complex (ABC) 1:100** with **ABC Dilution Buffer**.

8.3.2 For each well to be used in the experiment prepare 100 µL, by adding 1 µL of **100X Avidin-Biotin-Peroxidase Complex (ABC)** to 99 µL ABC Dilution Buffer.

8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.4 1X Wash Buffer

8.4.1 Add 270 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.

8.4.2 Add the entire 30 mL contents of the **10X Wash Buffer** bottle to the water.

8.4.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.4.4 Store the **1X Wash Buffer at** room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.5 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.
• For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
• Clear samples by centrifugation as follows:

- **Cell culture supernatants** - Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
- **Serum** - Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.
- **Plasma** - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1,500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

9.2 Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range.

• Prepare 150 µL sample for each replicate to be assayed.
• Dilute samples with Sample Diluent Buffer.
• Mix diluted samples gently and thoroughly.
• Pipetting less than 2 µL is not recommended for optimal assay accuracy.
• Refer to the following table for recommended sample dilution guidelines based on the dynamic range of this kit:

Estimated Sample Target Concentration		Dilution Level	Sample Volume For Two Replicates	Sample Diluent Buffer For Two Replicates
High Concentration	20-200 ng/mL	1:100	1 µL	99 µL
Medium Concentration	2-20 ng/mL	1:10	10 µL	90 µL
Low Concentration	31.2-2,000 pg/mL	1:2	50 µL	50 µL
Very Low Concentration	≤ 31.2 pg/mL	1:2 or No Dilution	-	-

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for intra- and inter-assay reproducibility will be obtained when performing all incubation steps at 37°C as indicated below.

10.1 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the pre-coated well plate. At least two replicates of each standard, sample or blank is recommended.

10.2 Cover the plate with the well plate lid and incubate at 37°C for 90 minutes.

10.3 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.5 Add 100 µL of prepared **1X Biotinylated Anti-Human PDGF-AB Antibody to each well.**

10.6 Cover with the well-plate lid and incubate at 37°C for 60 minutes.

10.7 Wash plate 3 times with **1X Wash Buffer as** follows:

10.7.1 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.7.2 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.7.3 Add 300 µL of **1X Wash Buffer** to each assay well.

10.7.4 Incubate for 1 minute.

10.7.5 Repeat steps 10.7.1 through 10.7.4 **two** more times.

10.8 Add 100 µL of prepared **1X Avidin-Biotin-Peroxidase Complex (ABC) into** each well and incubate at 37°C for 30 minutes.

10.9 Wash plate 5 times with **1X Wash Buffer as** follows:

10.9.1 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.9.2 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.9.3 Add 300 µL of 1X Wash Buffer to each assay well.

10.9.4 Incubate for 1 minute.

10.9.5 Repeat steps 10.9.1 through 10.9.4 **four** more times.

10.10 Add 90 µL of **TMB Color Developing Agent** to each well and incubate at 37°C in the dark for 15-25 minutes.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.11 Add 100 µL of **TMP Stop Solution** to each well. Well color should change to yellow immediately.

10.12 Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.11.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

(Relative OD_{450}) = (Well OD_{450})–(Mean Blank Well OD_{450})

The standard curve is generated by plotting the mean replicate Relative **OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The Human PDGF-AB concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

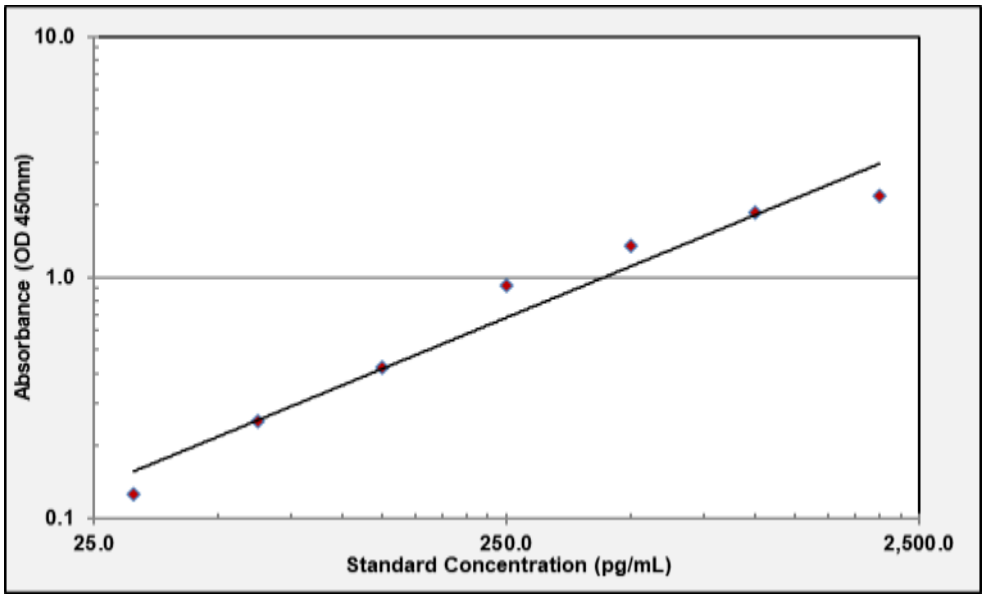
Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

12.1 Typical absorbance values. Expected absorbance for standards when TMB incubation is performed for 20 minutes at 37°C and measured at OD_{450}.

Standard Number	8	7	6	5	4	3	2	1
standard Concentration (pg/mL)	0	31.2	62.5	125	250	500	1,000	2,000
OD_{450}	0.023	0.126	0.252	0.423	0.922	1.358	1.856	2.195

12.2 Typical standard curve. This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



12.3 Reproducibility

	Intra-Assay			Inter-Assay		
Sample ID	1	2	3	1	2	3
n =	16	16	16	24	24	24
Mean Measured Concentration (pg/mL)	60	259	808	62	271	865
Standard Deviation	3.96	14.76	51.71	4.52	19.51	68.33
Consistency (%CV)	6.6	5.7	6.4	7.3	7.2	7.9

13. Technical Resources

13.1 References

13.1.1 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.

13.1.2 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.

13.1.3 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.

13.1.4 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.

13.1.5 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.

13.2 Technical Support

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