Version 1 Last updated 27 January 2017

ab218297
Porcine PDGF-BB ELISA Kit

For the quantitative measurement of porcine PDGF-BB in serum, plasma and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

The Porcine PDGF-BB ELISA kit (ab218297) is an in vitro enzyme-linked immunosorbent assay (ELISA) for the quantitative measurement of porcine PDGF-BB in serum, plasma and cell culture supernatants.

This assay employs an antibody specific for porcine PDGF-BB coated on a 96-well plate. Standards and samples are pipetted into the wells and PDGF-BB present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-porcine PDGF-BB antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of PDGF-BB bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 100 µL standard or sample to each well. Incubate 2.5 hours at room temperature.

Add 100 µL prepared biotin antibody to each well. Incubate 1 hour at room temperature.

Add 100 µL prepared Streptavidin solution. Incubate 45 minutes at room temperature.

Add 100 µL TMB Substrate Reagent to each well. Incubate 30 minutes at room temperature.

Add 50 µL Stop Solution to each well. Read at 450 nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All ELISA kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store ELISA kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- ELISA kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-Human PDGF-BB coated Microplate (12 x 8 wells)	1x 96 wells	-20°C
5X Assay Diluent	15 mL	-20°C
Lyophilized (PDGF-BB) Standard	2 Vials	-20°C
800X Streptavidin-Conjugated HRP	200 µL	-20°C
Biotinylated (PDGF-BB) Detection Antibody	2 vials	-20°C
20X Wash Buffer	25 mL	-20°C
TMB Substrate	12 mL	-20°C
Stop Solution	8 mL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution or sample dilutions.
- 100 mL and 1 liter graduated cylinders.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Anti-Human PDGF-BB coated Microplate (12 x 8 wells):
One plate of 96 wells. Ready to use. Store at -20°C.

9.2 5X Assay Diluent (15 mL):
Diluted 5X Assay Diluent 5-fold with deionized or distilled water before use.

9.3 Lyophilized (PDGF-BB) Standard (2 x 1 vials):
Briefly spin the vial. Add 400 µL 1X Assay Diluent to prepare a 50 ng/mL stock solution. Dissolve the powder thoroughly by a gentle mix.

9.4 800X Streptavidin-Conjugated HRP (200 µL):
Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 800-fold with 1X Assay Diluent E.g. Add15 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent to prepare an 800-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

9.5 Biotinylated (PDGF-BB) Detection Antibody (2 x 1 vials):
Briefly spin the Detection Antibody vial before use. Add 100 µL of 1X Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent.

9.6 20X Wash Buffer (25 mL):
If the Wash Concentrate (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.7 TMB Substrate
12 mL. Ready to use. Store at -20°C.

9.8 Stop Solution
8 mL. Ready to use. Store at -20°C.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 To prepare standards, label 8 Eppendorf tubes with #1 - #8.
10.2 Add 25 µL PDGF-BB stock solution into a tube with 475 µL 1X Assay Diluent to prepare a 2,500 pg/mL Standard #1.
10.3 Aliquot 300 µL of 1X Assay Diluent into tubes #2 - #8.
10.4 Transfer 200 µL from tube #1 to tube #2 and mix. Transfer 200 µL from tube #2 to tube #3 and mix, and so on until tube #7
10.5 Standard #8 contains no protein and is the Blank control.

Tube #	Volume to dilute	Volume of Assay Diluent	Concentration (pg/mL)
1	25 µL of 50 ng/mL stock solution	475 µL	2,500
2	200 µL of tube #1	300 µL	1,000
3	200 µL of tube #2	300 µL	400
4	200 µL of tube #3	300 µL	160
5	200 µL of tube #4	300 µL	64
6	200 µL of tube #5	300 µL	25.6
7	200 µL of tube #6	300 µL	10.24
8	N/A	300 µL	0

11. Sample Preparation

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.

- Dilute Serum 2-20 fold in 1X Assay Diluent.
- Dilute Plasma 2-20 fold in 1X Assay Diluent.
- Dilute Cell culture supernatant 2-20 fold in 1X Assay Diluent.

ΔNote: Levels of PDGF-BB may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Label removable 8-well strips as appropriate for your experiment.

12.2 Add 100 µL of each standard and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.

12.3 Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

12.4 Add 100 µL of 1X prepared biotinylated antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.

12.5 Discard the solution. Repeat the wash as in step 12.3.

12.6 Add 100 µL of prepared Streptavidin solution to each well. Incubate for 45 minutes at room temperature with gentle shaking.

12.7 Discard the solution. Repeat the wash as in step 12.3.

12.8 Add 100 µL of TMB Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

12.9 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

13. Calculations

13.1 Calculate the mean absorbance for each set of duplicate standards, controls and samples.
13.2 Subtract the average zero standard optical density.
13.3 Plot the standard curve on log-log, with standard concentration on the x-axis and absorbance on the y-axis.
13.4 Draw the best-fit straight line through the standard points.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.

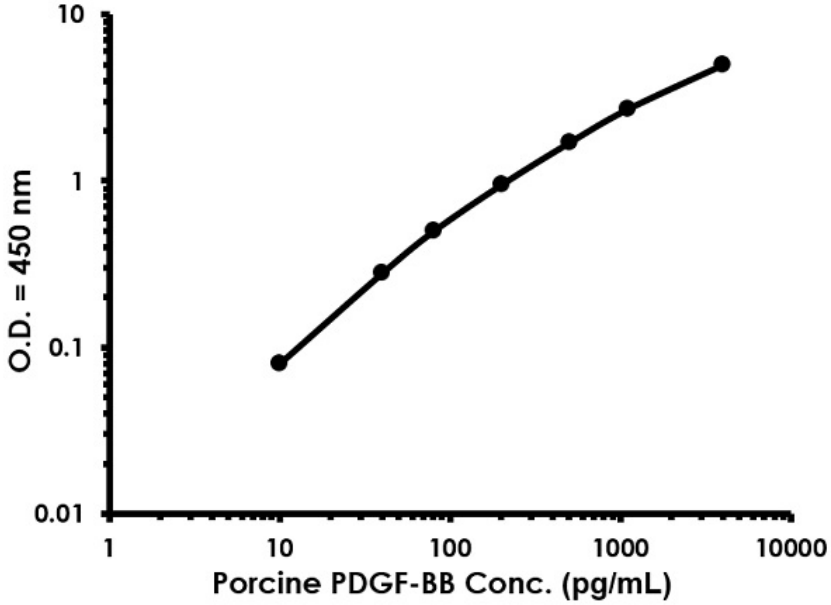

Figure 1. Porcine PDGF-BB ELISA kit (ab218297) Standard curve.

15. Typical Sample Values

SENSITIVITY –

The minimum detectable dose of Porcine PDGF-BB was determined to be 10 pg/mL.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

SPIKING & RECOVERY –

Recovery was determined by spiking various levels of Porcine PDGF-BB into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	108.6	87-123
Plasma	112.3	98-115
Cell culture media	120.5	108-130

LINEARITY–

Sample Type (1:2)	Average % of Expected	Range (%)
Serum	120	115-125
Plasma	106	88-117
Cell culture media	99.64	96-103

Sample Type (1:4)	Average % of Expected	Range (%)
Serum	112.4	106-119
Plasma	106.9	84-132
Cell culture media	105.9	91-121

REPRODUCIBILITY–

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

SPECIFICITY–

This ELISA antibody pair detects porcine and human PDGF-BB. Other species not determined.

16. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time. Sample and standard addition may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background).
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your standard at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light.
	Stop solution	Add stop solution to each well before reading plate

17. Notes

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