PDGFB (Human) ELISA Kit

Catalog Number KA1760

96 assays

Version: 02

Intended for research use only

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Introduction

Background

PDGFB (Human) ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human PDGF-BB cell lysate and tissue lysate. This assay employs an antibody specific for human PDGFBB 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-human 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.

General Information

Materials Supplied

Component	Amount
PDGF-BB Microplate (Item A): 96 wells coated with anti-human PDGF-BB.	96 (8x12) wells
Wash Buffer Concentrate (20X) (Item B): 20X concentrated solution.	25 mL
Standard Protein (Item C): Human PDGF-BB. 1 vial is enough to run each standard in duplicate.	2 vials
Detection Antibody PDGF-BB (Item F): Biotinylated anti-human PDGF-BB (each vial is enough to assay half microplate).	2 vials
HRP-Streptavidin Concentrate (Item G): 800X concentrated HRP-conjugated streptavidin.	200 µL
TMB One-Step Substrate Reagent (Item H): 3, 3', 5, 5'- tetramethylbenzidine (TMB) in buffered solution.	12 mL
Stop Solution (Item I): 0.2 M sulfuric acid.	8 mL
Sample Diluent Buffer (Item D2): 5X concentrated buffer.	10 mL
Assay Diluent (Item E2): 5X concentrated buffer.	15 mL
Cell lysate buffer (Item J): 2X cell lysate buffer.	5 mL

Storage Instruction

May be stored for up to 6 months at 2º to 8ºC from the date of shipment. Opened Microplate Wells or reagents may be store for up to 1 month at 2º to 8ºC. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. Reconstituted standard can be stored at -80ºC for up to 1 week.

Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

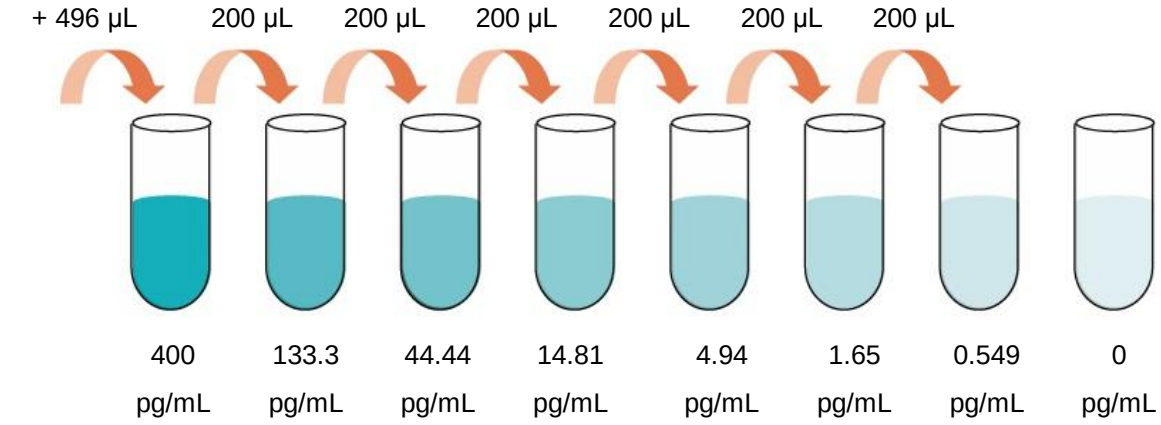
Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Precision pipettes to deliver 2 µL to 1 mL volumes.
- ✓ Adjustable 1-25 mL pipettes for reagent preparation.
- ✓ 100 mL and 1 liter graduated cylinders.
- ✓ Absorbent paper.
- ✓ Distilled or deionized water.
- ✓ Log-log graph paper or computer and software for ELISA data analysis.
- ✓ Tubes to prepare standard or sample dilutions.

Assay Protocol

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Sample Diluent Buffer (Item D2) and Assay Diluent (Item E2) should be diluted 5-fold with deionized or distilled water before use. Cell lysate buffer (Item J) should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).
3. Sample dilution: Tissue lysate and cell lysate samples should be diluted at least 5-fold with 1X Sample Diluent Buffer (Item D2). Generally we recommend a minimum of 1 mg of protein per 1 mL of original lysate solution, though more concentrated is better. We also recommend the addition of protease inhibitors (not included) to the lysis buffer prior to use.
   Note: Levels of PDGF-BB may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
4. Preparation of standard: Briefly spin the vial of Item C. Add 280 µL 1x Sample Diluent Buffer (Item D2) into Item C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 4 µL PDGF-BB standard from the vial of Item C, into a tube with 496 µL Sample Diluent Buffer to prepare a 400 pg/mL stock standard solution. Pipette 400 µL 1x Sample Diluent Buffer into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Sample Diluent Buffer serves as the zero standard (0 pg/mL).



5. If the Wash Concentrate (20x) (Item B) 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.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent (Item E2) 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 and used in step 4 of Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) 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 (Item E2).
   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 20 µL of HRP-Streptavidin concentrate into a tube with 16 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.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µL of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
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 autowasher. 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.
4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µL standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.
3. Add 100 µL prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µL prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µL TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µL Stop Solution to each well. Read at 450 nm immediately

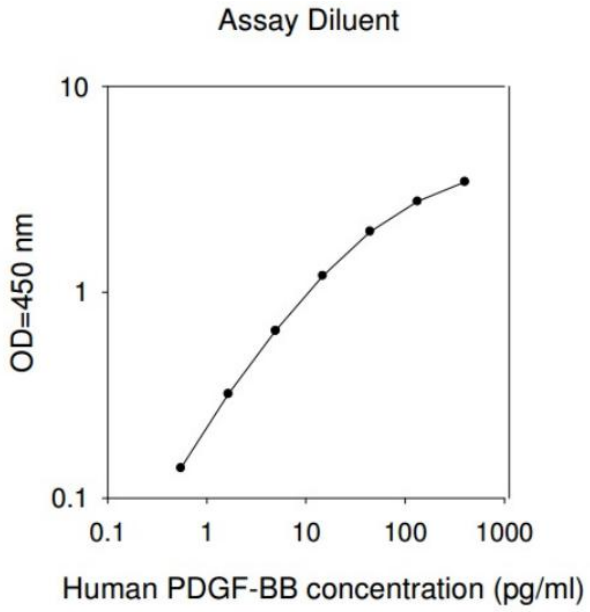
Data Analysis

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical data

These standard curves are for demonstration only. A standard curve must be run with each assay.



Performance Characteristics

- Sensitivity

  The minimum detectable dose of Human PDGF-BB was determined to be 1 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 Human PDGF-BB into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Tissue lysate	92.78	82-104
Cell lysate	94.37	83-105

- Linearity

Sample Type		Tissue lysate	Cell Lysate
1:2	Average % of Expected	93	92
	Range (%)	82-103	84-104
1:4	Average % of Expected	94	97
	Range (%)	83-104	83-103

- Reproducibility

  Intra-Assay: CV<10%

  Inter-Assay: CV<12%

- Specificity

  The antibody pair provided in this kit recognizes human PDGF-BB.

Resources

Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Briefly centrifuge Item C and dissolve the powder thoroughly by gently 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; assay procedure step 2 change may be done overnight.
	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.

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H