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Innovative Plate Assay Solutions

# Human PDGF-BB ELISA

**Catalog Number EA-0404** **(For Research Use Only)**

## Introduction

Platelet-derived growth factor (PDGF) is a growth factor that is important to embryonic development, cell proliferation, and cell migration, particularly, in blood vessel formation (angiogenesis). Like VEGF, PDGF is able to independently initiate angiogenesis and mediate blood vessel growth and behavior. The protein is a dimeric glycoprotein composed of two A (-AA) or two B (-BB) chains or a combination of the two (-AB). It has been shown that the cis oncogene is derived from the PDGF B-chain gene. It binds to PDGF receptors, which activate signal transduction pathways such as the PI3K pathway, and subsequently regulate downstream gene expression and the cell cycle. PDGF has also been linked to several diseases such as atherosclerosis, fibrosis and malignant diseases.

## Principle of the assay

PDGF ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes rabbit anti-human PDGF antibodies for immobilization on the microtiter wells and rabbit anti-human PDGF antibodies along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the PDGF molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of PDGF is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

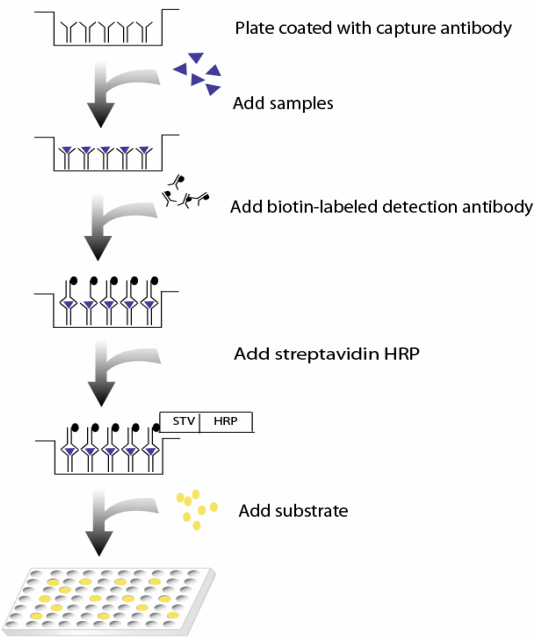



Diagram of ELISA

## Materials provided with the kit

- 96 well microplate coated with rabbit anti-human PDGF antibodies (4°C).
- Biotin labeled rabbit anti-human PDGF antibodies (9µg/ml) (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Recombinant human PDGF standard (-20°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer (RT)
- Substrate (4°C).
- Stop Solution (4°C).

## Material required but not provided

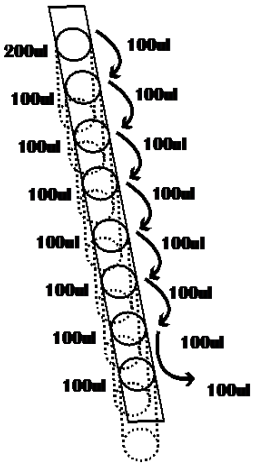
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

## Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 100 times of human recombinant PDGF-BB (400ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions. To dilute 100 times of Human PDGF-BB, add 2ul Human Recombinant PDGF-BB in 200ul 1X Diluent Buffer (See Step 2 in "Assay Procedure" for detailed instruction)
- Dilute 400 times of biotin labeled rabbit anti-human PDGF-BB with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

## Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed.
2. See instruction and diagram below for standard preparation.




a. Add 200ul 1X Diluent buffer to the 1st well. Add 100ul 1X Diluent Buffer to the rest wells of strip.
b. Add appropriate amount of protein recombinant (follow instruction in "Reagent Preparation")
c. Mix dilutions in 1st well and transfer 100ul from the 1st well to the next dilution. (See picture) Incubate each well for 1 hr at room temperature with gentle shaking

3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
4. Add 100µl of diluted biotin-labeled goat anti-human PDGF-BB antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Add 100µl substrate to each well and incubate for 10-30 minutes.
9. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

## Example of standard curve