CELL APPLICATIONS, INC.

Rat PDGF-AB ELISA Kit

Cat. No. CL0485
96-wells

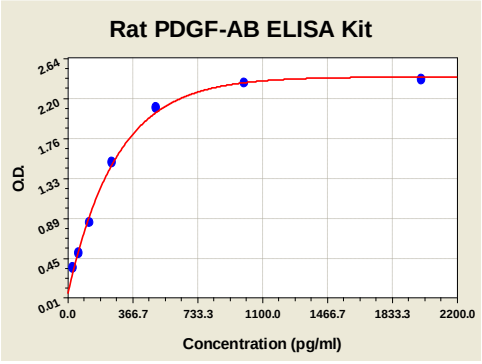
Target Protein Species: Rat
Range 31.2pg/ml – 2000pg/ml
Specificity No detectable cross-reactivity with any other cytokine

KIT COMPONENTS

Component	Amount
96-well plate precoated with anti-Rat PDGF-AB antibody	1 Plate
Protein Standard: Lyophilized recombinant Rat PDGF-AB	2 tubes, 10 ng/tube
Sample Diluent Buffer	30 ml
Biotinylated Antibody (Anti-Rat PDGF-AB)	130 µl (100x)
Antibody Diluent Buffer	12ml
Avidin-Biotin-Peroxidase Complex (ABC) Solution	130 µl (100x)
ABC Diluent Buffer	12 ml
Tetramethylbenzidine (TMB) Color Developing Agent	10 ml
TMB Stop Solution	10 ml

Storage

Store at 4°C. Cell Applications, Inc. recommends using the kit within 6 months of order.



X	pg/ml	0.0	31.2	62.5	125	250	500	1000	2000
Y	O.D.450	0.084	0.342	0.508	0.842	1.501	2.102	2.366	2.410

Figure 1: PDGF-AB Standard Curve. Using the Rat PDGF-AB ELISA Kit, O.D. data was graphed against PDGF-AB protein concentration. The TMB reaction was incubated at 37° C for 8 min.

BACKGROUND

Platelet-derived growth factor (PDGF) is a major mitogen for connective tissue cells and certain other cell types. It is a dimeric molecule consisting of disulfide-bonded, structurally similar A- and B-polypeptide chains, which combine to homo- and heterodimers. The PDGF isoforms exert their cellular effects by binding to and activating two structurally related protein tyrosine kinase receptors, denoted the alpha-receptor and the beta-receptor. The PDGF alpha-receptor binds all three isoforms with high affinity whereas the beta-receptor binds only PDGF-BB with high affinity, PDGF-AB with low affinity and does not appear to bind PDGF-AA. Ligand binding induces receptor dimerization and autophosphorylation. This enables a number of SH2 domain containing signal transduction molecules to bind to the receptors, thereby initiating various signaling pathways.[1] Moreover, binding of PDGF to its receptor is followed by internalization and degradation of the ligand-receptor complex. Experiments with mutant receptors have shown that ligand-induced internalization is not absolutely dependent on the kinase activity of the beta-receptor.[2]

Activation of PDGF receptors leads to stimulation of cell growth, but also to changes in cell shape and motility; PDGF induces reorganization of the actin filament system and stimulates chemotaxis, i.e., a directed cell movement toward a gradient of PDGF. In vivo, PDGF isoforms have important roles during the embryonic development, particularly in the formation of connective tissue in various organs. In the adult, PDGF stimulates wound healing. Moreover, overactivity of PDGF has been implicated in several pathological conditions. The sis oncogene of simian sarcoma virus (SSV) is related to the B-chain of PDGF, and SSV transformation involves autocrine stimulation by a PDGF-like molecule. Similarly, overproduction of PDGF may be involved in autocrine and paracrine growth stimulation of human tumors. Overactivity of PDGF has, in addition, been implicated in nonmalignant conditions characterized by an increased cell proliferation, such as atherosclerosis and fibrotic conditions.[3] Different kinds of PDGF antagonists are currently being developed and evaluated in different animal disease models, as well as in clinical trials.

Reference

1. Westermark, B. et al: Ciba Found Symp.150:6-14, 1990
2. Heldin, C.H. & Westermark, B.: Physiol Rev. 79:1283-316, 1999
3. Ostman, A. & Heldin, CH.: Adv Cancer Res. 80:1-38, 2001

ELISA OVERVIEW

Cell Applications ELISA Kits are based on standard sandwich enzyme-linked immunosorbent assay technology. Freshly prepared standards, samples, and solutions are recommended for best results.

1. Prepare test samples.
2. Prepare a protein standard of the target protein.
3. Add test samples and standards to the pre-coated 96-well plate. Do not wash.
4. Add biotinylated detection antibodies. Wash.
5. Add Avidin-Biotin-Peroxidase Complex (ABC) Solution. Wash.
6. Add Tetramethylbenzidine (TMB) Color Developing Agent, containing HRP substrate.
7. Add TMB Stop Solution
8. Subject the plate to analysis.

NOTES:

- Before using the kit, quick spin tubes to bring down all solution to the bottom of tube.
- Duplicate assay wells are recommended for both standard and sample testing.
- Do not let the 96-well plate dry, this will lead to inactivation of plate components.
- When diluting samples and reagents, ensure that they are mixed completely and evenly.
- Pre-warm diluted ABC and TMB solutions at 37°C for 30 min before use to avoid variable temperature effects.
- For washes, use TBS or PBS. Do not touch well walls.
- A protein standard is included in the kit. A protein standard detection curve should be generated with each experiment, no more than 2 hours prior to the experiment.
- The user will determine sample dilution fold by estimation of target protein amount in samples.

ELISA PROTOCOL

Preparation of Test Samples

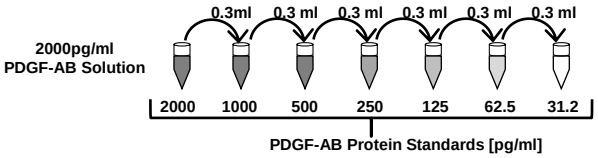
1. Process Test Samples in the following manner:
   - **Cell culture supernate, tissue lysate or body fluids**: Remove particulates by centrifugation. analyze immediately or aliquot and store at -20° C
   - **Serum**: Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 1500 x g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20° C.
   - **Plasma:** Collect plasma using heparin, EDTA as an anticoagulant. Centrifuge for 15 min at 1500 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. Estimate the concentration of the target protein in the sample and select a proper dilution factor such that the diluted target protein concentration falls within the 31.2-2000 pg/ml standard curve range. Depending on the sample, several trial dilutions may be necessary. Dilute the sample using the provided diluent buffer, mixing well. Suggested working dilutions of samples are as follows:

Target Protein Concentration Range	Sample Working Dilution	Sample Vol.	Diluent Buffer Vol.
20-200ng/ml	1:100	1 µl	99 µl
2-20 ng/ml	1:10	10 µl	90 µl
31.2-2000 pg/ml	1:2	50 µl	50 µl
≤31.2 pg/ml	n/a	100µl	n/a

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

Preparation of Standard Solutions (31.2-2000 pg/ml)

4. Reconstitute the Lypophilized Recombinant Protein to make a 10.000 pg/ml Rat PDGF-AB solution. Add 1 ml Sample Diluent Buffer to a tube of lypophilized protein, keep the tube at room temperature for 10 min. Mix thoroughly.
5. Add 0.2 ml of the mixed 10,000 pg/ml PDGF-AB solution to the eppendorf tube containing 0.8 ml diluent buffer and mix to make a 2000 pg/ml PDGF-AB solution
6. Label 6 eppendorf tubes with the PDGF-AB protein concentrations to be prepared by serial dilution: 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml.
7. Aliquot 0.3 ml of the Sample Diluent Buffer to the labeled tubes.
8. Serially dilute the protein standards into their respectively labeled tubes. Transfer 0.3 ml from the 2000pg/ml PDGF-AB Solution to the 1000pg/ml eppendorf tube and mix thoroughly. Transfer 0.3 ml of the 1000 pg/ml solution to the 500pg/ml tube and mix thoroughly, Transfer 0.3 ml of the 500pg/ml solution to the 250pg/ml tube and mix, and so on to make the 125, 62.5 and 31.2 pg/ml solutions.
9. Store at 4° C until use.



Loading the 96-well Plate

10. Aliquot 0.1 ml of the sample diluent buffer into a control well to serve as the Blank. This will yield the O.D.450(Blank) reading.
11. Aliquot 0.1 ml of the standard solutions of the **Preparation of Standard Solutions** (31.2-2000pg/ml) into empty wells of the precoated 96-well plate. Duplicate measurements of standards are recommended.
12. Aliquot 0.1 ml of each properly diluted test sample to empty wells prepared in **Step 2**. Duplicate measurements of each test sample are recommended.
13. Cover the 96-well plate and incubate at 37° C for 90 min.
14. During the **Step 13** incubation period, prepare a stock of Biotinylated 1:100 Antibody Working Solution. Count the number of reactions and multiply by 0.1 ml/well for the Working Solution total volume (preparation of 1-2 reactions in excess of the number of wells is recommended). Dilute the Biotinylated Antibody to 1:100 in Antibody Diluent Buffer and mix thoroughly. Use the working solution within 2 hours.
15. Upon completion of the 90 min incubation of **Step 13**, remove the cover of the 96 well plate and discard plate well contents. Blot the plate onto paper towels or other absorbent material. DO NOT let the wells completely dry at any time.
16. Add 0.1 ml of the Biotinylated 1:100 Antibody Working Solution (prepared in **Step 14**) to each well and incubate the plate at 37° C for 60 min.
17. During the incubation period of **Step 16**, prepare a stock of ABC Working Solution. Count the number of reactions and multiply by 0.1 ml/well for the Working Solution total volume (preparation of 1-2 reactions in excess of the number of wells is recommended). Dilute the ABC Stock Solution to 1:100 in ABC Diluent Buffer and mix thoroughly. Pre-warm the ABC working solution at 37° C for 30 min before use. Use the working solution within 1 hour.
18. Upon completion of the 60 min incubation of **Step 16**, wash the plate 3 times with 0.3 ml TBS or PBS. For each wash, leave washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
19. Add 0.1 ml of prepared ABC Working Solution (prepared in **Step 17**) to each well and incubate the plate at 37° C for 30 min.
20. During the incubation period of **Step 19**, pre-warm TMB Color Developing Agent at 37° C for 30 min before use.
21. Upon completion of the 30 min incubation of **Step 19**, wash the plate 5 times with 0.3 ml TBS or PBS. For each wash, leave the washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
22. Add 90 µl of the pre-warmed TMB Color Developing Agent into each well and incubate at 37° C for 6-10 min (shades of blue can be seen in the wells with the four most concentrated Protein Standard Solutions; the other control wells should show no obvious color).
23. Add 0.1 ml of the TMB Stop Solution to each well. The acidic stop solution will change the mixture color to yellow. The yellow intensity is proportional to the amount of target protein captured by the plate.
24. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution. These readings are the O.D.450(Reading).

Calculating Protein Concentration

- For all wells, determine O.D.450(Relative):
  O.D.450(Relative) = O.D.450(Reading) – O.D.450(Blank)
- Plot the standard curve: plot O.D.450(Relative) of each standard solution (Y) vs. the respective concentration of the standard solution (X). See **Figure 1** for a typical standard curve.
- The Rat PDGF-AB concentration of the samples can be interpolated from the standard curve. Multiply the interpolated concentration by the dilution factor to obtain the target protein concentration in the sample.