BioAim Scientific Inc

Human PDGF-AB ELISA Kit

Cat.No: 3010125

Instruction Manual (Last revised April 10, 2017)

For research use only

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I. INTRODUCTION

The Bioaim Human PDGF-AB ELISA kit is a solid phase sandwich ELISA (enzyme-linked immunosorbent assay) for the quantitative measurement of PDGF-AB in human serum, plasma and cell culture media. An antibody specific for human PDGF-AB was coated on a 96-well plate. Standards and samples are added to the wells and any PDGF-AB present binds to the immobilized antibody. The wells are washed and biotinylated anti-human PDGF-AB antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is added to the wells. The wells are again washed and TMB substrate solution is added, which produces a blue color in direct proportion to the amount of PDGF-AB present in the initial sample. The Stop Solution changes the color from blue to yellow, and the microwell absorbances are read at 450 nm.

II. REAGENTS

1. Human PDGF-AB Microplate: 96 breakable wells (12strips x 8wells) coated with anti-human PDGF-AB.
2. 20x Wash Buffer Concentrate: 1 Vial, 25 ml.
3. 5x Assay Diluent: 1vial, 15 ml.
4. Standards: 2 vials, recombinant human PDGF-AB.
5. Detection Antibody: 2 vials, biotinylated anti-human PDGF-AB.
6. HRP-Streptavidin Concentrate: 1vial.
7. TBM Substrate solution: 1 Vial, 12 ml.
8. Stop Solution: 1 Vial, 8 ml of 0.2 M sulfuric acid.

III. STORAGE

1. The entire kit may be stored at -20°C for up to 1 year from the date of shipment. For extended storage, it is recommended to store at -80°C. The kit can be stored for up to 6 months at 2º to 8ºC from the date of shipment.
2. Standard can be stored at -20 ºC or -80 ºC. Use freshly prepared standard within 12 hours (stored at 2~8 ºC).
3. Opened Microplate Wells or reagents may be stored for up to 1 month at 2 to 8 ºC. Return unused strips to the pouch containing desiccant pack, reseal along entire edge and keep in 2~8 ºC.
4. Avoid repeated freeze-thaw cycles.

IV. ADDITIONAL MATERIALS REQUIRED

1. Distilled or deionized water.
2. Precision pipettes, with disposable plastic tips.
3. Beakers, flasks, cylinders necessary for preparation of reagents.
4. Microplate washing device (multichannel pipette or automated microplate washer).
5. Microplate shaker.
6. Microplate reader capable of reading at 450 nm.

V. PRECAUTIONS

1. All reagents must be at room temperature (18 ºC to 25 ºC) before running assay.
2. Do not mix or substitute reagents with those from other lots or other sources.
3. Do not use kit reagents beyond expiration date on label.
4. Do not expose kit reagents to strong light during storage or incubation.
5. Use disposable pipette tips for each transfer to avoid microbial contamination or cross contamination of reagents.
6. Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results.
7. Avoid contact of stop solution with skin or eyes. If contact occurs, immediately flush area with copious amounts of water.
8. Do not use TMB substrate solution if it has begun to turn blue.
9. Do not expose bleach to work area during actual test procedure because of potential interference with enzyme activity.

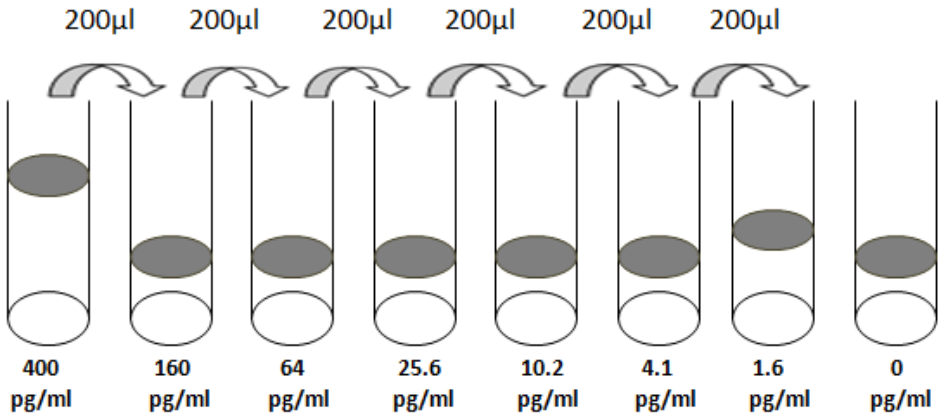
VI. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18~25°C) before use.
2. **Assay diluent**
   Dilute the concentrated assay diluent 1:5 with distilled water (e.g. 10ml plus 40ml).
3. **Wash Buffer**
   Dilute the concentrated wash buffer 1:20 with distilled water (e.g. 20ml plus 380ml).
4. **Sample**
   Levels of the target protein may vary among different specimens. Optimal dilution factors for each sample must be determined by the investigator.

The dilution scheme is only suggestion: the recommended dilution for serum and plasma is 1:50~200.

5. Standard

a. Briefly spin standard vial before use. Add 200 µl 1x Assay Diluent to prepare a 5ng/ml standard. Gently vortex to mix.
b. Take 40 µl standard into a tube; then add 460 µl 1x Assay Diluent to prepare a 400 pg/ml stock standard solution.
c. Add 300 µl 1x Assay Diluent to 7 tubes. Label as 160pg/ml, 64pg/ml, 25.6pg/ml, 10.2pg/ml, 4.1pg/ml, 1.6pg/ml and the last tube with 1x assay diluent is the blank as 0pg/ml.
d. Perform serial dilutions by adding 200 µl of each standard to the next tube and vortexing between each transfer (see figure below).



6. Detection Antibody

Briefly spin the Detection Antibody vial before use. Add 100 µl of 1X Assay Diluent into the vial to prepare a detection antibody concentrate. The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent and used in step 5 of Part VII Assay Procedure.

7. HRP-Streptavidin concentrate

Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 500-fold with 1X Assay Diluent.

For example: Add 20 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1X Assay Diluent to prepare a final 500 fold diluted HRP-Streptavidin solution. Mix well.

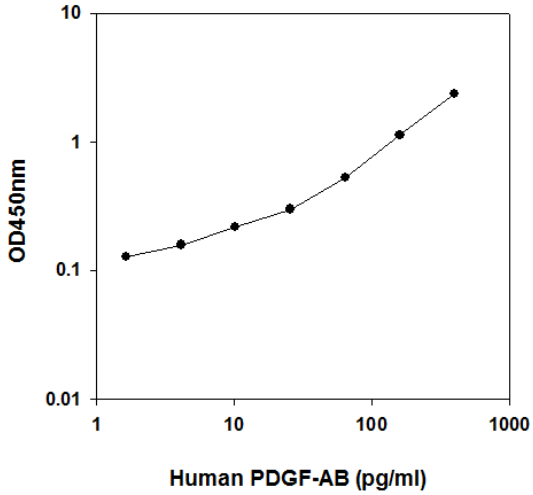
VII. ASSAY PROCEDURE

1. All reagents must be brought to room temperature (18-25°C) prior to use. It is recommended that all samples, standards, and blanks be run in duplicate.
2. Remove required quantity of test strips/wells, place in well holder.
3. Add 100 µl of each standard (*see Reagent Preparation step 5*) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature or over night at 4ºC with gentle shaking.
4. Decant or aspirate contents of wells. Wash wells by filling with at least 300 µl/well prepared wash buffer followed by decanting/aspirating. Soak wells in wash buffer for 30 seconds to 1 minute for each wash. Repeat wash 4 times for a total of 5 washes. After the last wash, blot plate on absorbent paper to remove residual buffer. Thorough washing at this step is very important, complete removal of liquid is required for proper performance.
5. 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.
6. Discard the solution. Repeat the wash as in step 4.
7. 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.
8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately. Read absorbance at 450nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract the optical density readings at 570nm from readings at 450nm.

VIII. CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of duplicate standards, samples and controls. Subtract the average zero standard optical density.
2. Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the PDGF-AB concentration on log-log graph paper or using Sigma plot software. Draw a best fit curve through the points of the graph.
3. To determine the concentration of circulating PDGF-AB for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding PDGF-AB concentration.
4. Computer-based curve-fitting statistical software may also be employed.
5. A representative standard curve is shown below. This standard curve is for demonstration only. A standard curve must be run with each assay by operator.



IX. PERFORMANCE CHARACTERISTICS

A. Sensitivity

The minimum detectable dose of human PDGF-AB was determined to be 1pg/ml. This is defined as two standard deviations above the mean optical density of 20 replicates of the zero standards.

B. Recovery

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

Sample Type	Average % Recovery	Range %
Serum	79	75-86
Plasma	78	77-84
Cell culture media	80	77-88

C. Linearity

Sample	Dilution	% of expected
Seum	1:2	112
	1:4	88
	1:8	82
Plasma	1:2	102
	1:4	79
	1:8	87
Cell culture media	1:2	103
	1:4	93
	1:8	92

D. Specificity

No cross-reactivity was tested with the following cytokines: human Angiogenin, BDNF, BLC, BMP-4, BMP-6, ENA-78, FGF-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 p70, IL-12 p40, IL-13, IGFBP-1, IGFBP-2, IGFBP-4, MCP-1, MCP-2, MCP-3, MDC, MIP-1 alpha, , MMP-1, MMP- 2, MMP-3, MMP-9, MMP-10, PARC, RANTES, SCF, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, VEGF.

E. Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

X. Troubleshooting

Problem	Cause	Solution
1.Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. check pipettes; 2. Ensure briefly spin the vial of standard, take the right amount to dilution.
2. Low signal	1. Too brief incubation time 2. Inadequate reagent volumes or improper dilution	1. ensure adequate incubation time; 2. Check pipettes and ensure corrected preparation.
3. Large CV	Inaccurate pipetting	1. Check pipettes; 2. Accurately perform each step.
4.High background	1. Plate is insufficiently washed; 2. Wash buffer contamination	1.Follow the manual correctly; if using a plate washer, check that all ports are working functionally; 2. Prepare fresh buffer.
5. Low sensitivity	1.ELISA kit improper storage 2. Stop solution	1. Follow the manual to store each component correctly; 2. Add enough stop solution to each well.

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