PDGFRB (Human) ELISA Kit

Catalog Number KA2142

96 assays

Version: 02

Intended for research use only

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Introduction

Principle of the Assay

The PDGFRB (Human) ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human PDGFR-beta in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human PDGFR-beta coated on a 96-well plate. Standards and samples are pipetted into the wells and PDGFR-beta present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human PDGFR-beta 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 PDGFR-beta 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
PDGFR-beta Microplate (Item A): coated with anti-human PDGFR-beta antibody.	96 (8x12) wells
Wash Buffer Concentrate (20x) (Item B): 20x concentrated solution.	25 ml
Standards (Item C): recombinant human PDGFR-beta.	2 vials
Assay Diluent D (Item K): 5x concentrated buffer. For Standard/Sample (serum/plasma samples/cell culture medium/urine) diluent.	15 ml
Assay Diluent B (Item E): 5x concentrated buffer. For detection antibody and HRP-Streptavidin diluent.	15 ml
Detection Antibody PDGFR-beta (Item F): biotinylated anti-human PDGFR-beta (each vial is enough to assay half microplage).	2 vial
HRP-Streptavidin concentrate (Item G): 300x 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

Storage Instruction

May be stored for up to 6 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2o to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. *Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.*

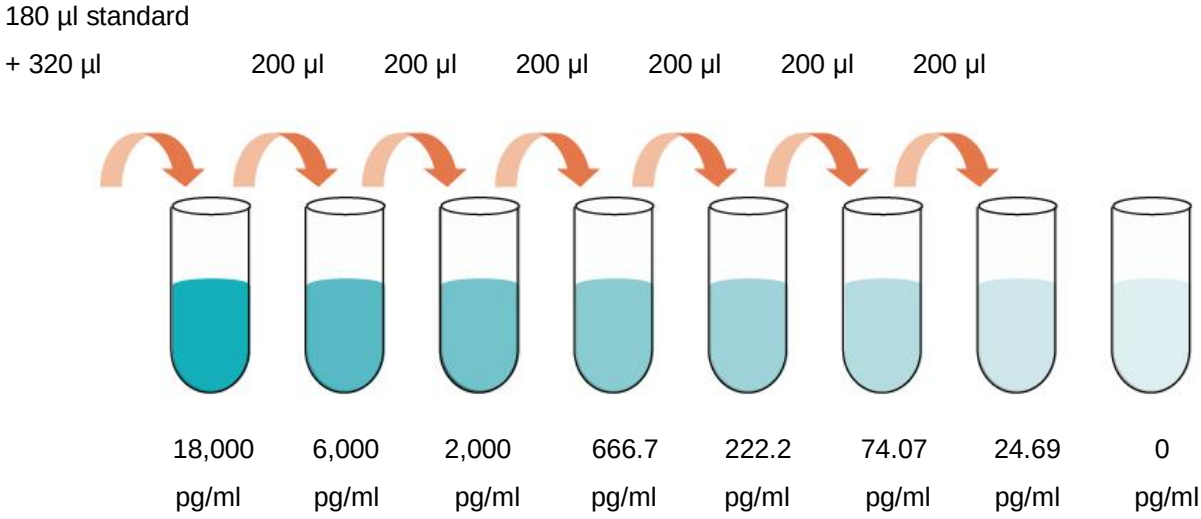
Materials Required but Not Supplied

- ✓ 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 sample dilutions.

Assay Protocol

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Sample dilution: If your samples need to be diluted, 1x Assay Diluent D (Item K) should be used for dilution of serum/plasma/culture/supernatants/urine.
   Suggested dilution for normal serum/plasma: 20-100 fold*
   *Please note that levels of the target protein may ary between different speciens. Optimal dilution factors for each sample must be determined by the inestigator.
3. Assay Diluent D (Item K) and Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use.
4. Preparation of standard: Briefly spin the vial of Item C. Add 400 µl 1x Assay Diluent D (Item K) into Item C vial to prepare a 50 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Add 180 µl PDGFR-beta standard from the vial of Item C, into a tube with 320 µl 1x Assay Diluent D to prepare a 18,000 pg/ml standard solution. Pipette 400 µl 1x Assay Diluent D into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Gently vortex to mix. 1x Assay Diluent D 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 B (Item E) 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 Band 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. HRP-Streptavidin concentrate should be diluted 300-fold with 1x Assay Diluent B (Item E).

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 40 µl of HRP-Streptavidin concentrate into a tube with 12 ml 1x Assay Diluent B to prepare a 300-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Assay Procedure

1. Bring all reagents to room temperature (18 - 25°C) before use. It is recommended that all samples or Positive Control should be run at least in duplicate.
2. Add 100 µl of each sample or positive control into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or over night at 4°C with 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 stip 7) to each well. Incubate for 45 minutes at room temperature with 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 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 sample or positive control to each well.
   Incubate 2.5 hours at room temperature or over night at 4°C.
3. Add 100 µl prepared primary antibody to appropriate well.
   Incubate 1 hours 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.

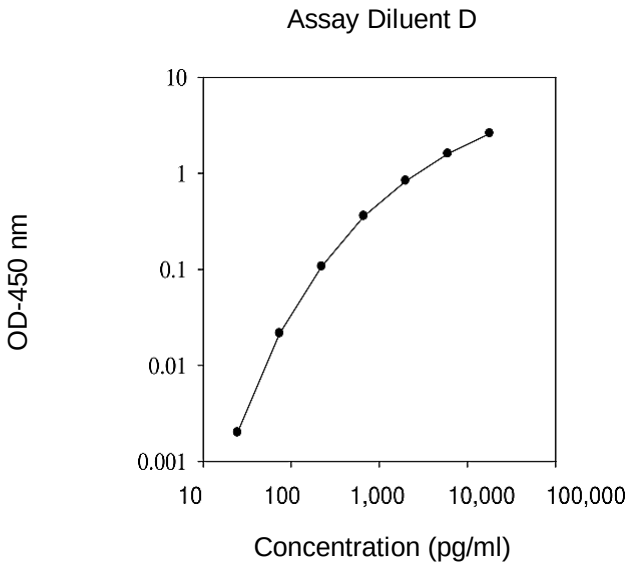
Data Analysis

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the aerage 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 PDGFR-beta is typically less than 25 pg/ml.

✓ Recovery

Recovery was determined by spiking various levels of PDGFR-beta into normal human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	109.6	97-118
Plasma	96.23	81-117
Cell culture media	90.39	76-104

✓ Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	113.3	101.7	103.7
	Range (%)	97-123	89-111	91-109
1:4	Average % of Expected	76.3	74.6	73.7
	Range (%)	65-86	64-86	61-83

✓ Reproducibility

Intra-Assay: CV<10%

Inter-Assay: CV<12%

✓ Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN-γ, Leptin (OB), MCP-1, MCP-3, MDC, MIP-1α, MIP-1 β, MIP-1δ, MMP-1, -2, -3, -10, PARC, RANTES, SCF, TARC, TGF-β, TIMP-1, TIMP-2, TNF-α, TNF-β, TPO, VEGF).

Resources

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 Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times 2. Inadequate reagent volumes or improper dilution	1. Ensure sufficient incubation time; assay procedure step 2 change to over night 2. Check pipettes and ensure correct preparation
3. Large CV	1. Inadequate reagent volumes or improper dilution	1. Check pipettes
4. High background	1. Plate is insufficiently washed 2. Contaminated wash buffer	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed. 2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the ELISA kit 2. Stop solution	1. Store your standard at < -20°C after reconstitution, other at 4°C. Keep substrate solution protected from light. 2. Stop solution should be added to each well before measurement and read OD immediately.

Plate Layout

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