PEDF (Human)

ELISA KIT PROTOCOL

(Catalog No.: EK-012-57)
(range: 2.2 - 140 ng/ml)

PHOENIX PHARMACEUTICALS, INC.

TABLE OF CONTENT

INTRODUCTION AND PROTOCOL OVERVIEW

The Pigment epithelium-derived factor (PEDF) was originally discovered as a secreted factor produced by the retinal pigmented epithelium (RPE), a layer of cells that supports the retina. Upon noticing RPE produced a factor that promoted the differentiation of primitive retinal cells into cells of a neuronal phenotype, this neurotrophic protein around 50 kilodaltons (kDa) was identified. The PEDF protein is a member of the serpin (serine protease inhibitor) family. Expression of PEDF in the human retina is found at 7.4 weeks of gestation, suggesting it may play a role in retinal neuron differentiation.

Phoenix Pharmaceutical's PEDF (Human) ELISA Kit is designed to measure the concentration of PEDF from human serum/plasma, or conditioned medium.

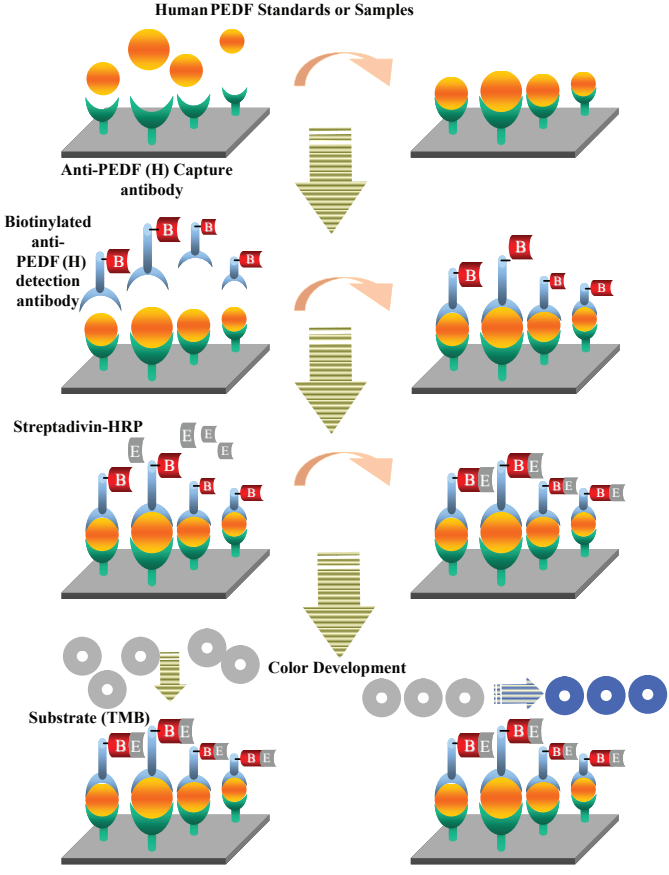
The immunoplate in this kit is precoated with anti-PEDF Capture Antibody and the nonspecific binding sites are blocked. The Human PEDF in the sample or in the standard solution can bind to the capture antibody immobilized in the wells. After washing procedure, the Biotinylated anti-PEDF Detection Antibody which can bind to the PEDF trapped in the wells is added. After washing, the Streptavidin-Horseradish Peroxidase (SA-HRP) which catalyzes the Substrate Solution (TMB) is added. The enzyme-substrate reaction is terminated by the addition of a stop solution. The intensity of the the color is directly proportional to the amount of PEDF in the standard solutions or samples. A standard curve of PEDF with known concentration can be established accordingly. The PEDF with unknown concentration in samples can be determined by extrapolation to this standard curve.

ASSAY CONDITIONS

Plasma, serum, culture media, tissue homogenate, CSF, urine or any biological fluid can be assayed as long as the level of the sample is high enough the for the sensitivity of the kit to detect it.

CAUTION: Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

Assay Principle



LIST OF COMPONENTS

Store all components at 4°C. DO NOT FREEZE.

1. Wash Buffer Concentrate *(20x, 50ml)*.....**Catalog No. EK-BUF**
2. Assay Diluent (PBS and BSA) .....**Catalog No. EK-BUF-012-57** *(1x, 75ml)*
3. 96 Well anti-PEDF Capture.....**Catalog No. EK-Plate-012-57** Antibody-Coated Plate *(1 plate)*
4. PEDF (Human) Standard.....**Catalog No. EK-S-012-57** *(lyophilized powder, 0.5µg/vial)*
5. Biotinylated anti-PEDF.....**Catalog No. EK-D-012-57** Detection Antibody *(1 vial)*
6. PEDF (Human) Positive Control.....**Catalog No. EK-PC-012-57** *(2 vials)*
7. Streptavidin-Horseradish Peroxidase.....**Catalog No. EK-HRP** (SA-HRP) *(2000x, 15µl)*
8. Urea for sample preparation..... **Catalog No. EK-UREA** *(0.48g/vial, 2 vials)*
9. Substrate Solution (TMB) *(12ml)*.....**Catalog No. EK-SS**
10. Stop Solution 2N HCl *(15ml)* .....**Catalog No. EK-HCL**
11. Acetate Plate Sealer (APS) *(3 pieces)*.....**Catalog No. EK-APS**
12. Assay Diagram *(1 sheet)*

MATERIALS REQUIRED BUT NOT SUPPLIED

- Micropipettor(s) and disposable pipette tips
- Multi-channel pipette capable of dispensing 50-100µl
- Solution Reservoir *(recommended)*
- Microtiter plate washer *(recommended)*
- Orbital plate shaker capable of 300-500 rpm *(recommended)*
- Microtiter plate reader capable of absorbance measurement 450nm
- Well-closed containers (15ml tubes or more in capacity)
- Absorbent material for blotting

REAGENT PREPARATION

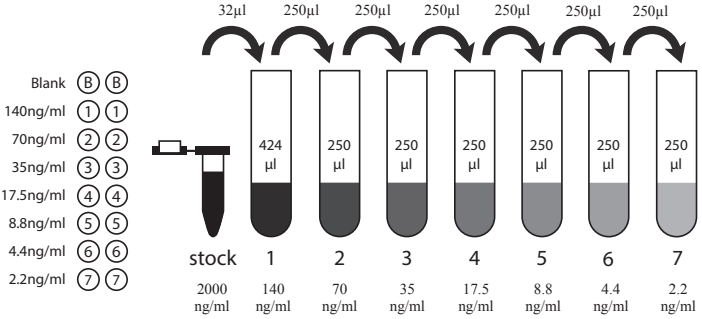
Note: *The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recome nended that the solutions be used as soon as possible after rehydra tion.*

1. **1x Wash Buffer:** Dilute the **20x** Wash Buffer Concentrate with 950ml of distilled water. If crystals are observed in the **20x** Wash Buffer, warm the bottle in a 37°C water bath for approximately 30 minutes or until the crystals disappear. After preparation, store **1x** Wash Buffer at 4°C. This buffer is used to wash the plate wells.
2. **Biotinylated anti-PEDF (Human) Detection Antibody:** Rehydrate Biotinylated anti-PEDF (Human) Detection Antibody with 100µl of **1x** Assay Diluent, vortex (centrifuge the tube to dislodge powder from the cap or walls). Further dilute Biotinylated anti-PEDF (Human) Detection Antibody to 1:100 as needed with Assay Diluent and mix thoroughly before use.
3. **Streptavidin-Horseradish Peroxidase (SA-HRP):** Centrifuge the HRP vial (15µl) provided in this kit (3,000-5,000 rpm, 5 seconds) and dilute HRP with **1x** Assay Diluent to 1:2000 before use. Vortex thoroughly.
4. **Human PEDF Positive Control:** Rehydrate Human PEDF Positive Control with 420µl of **1x** Assay diluent (centrifuge the tube to dislodge powder from cap or walls). Vortex thoroughly.
5. **Urea for 8M Solution:** Each vial contains 0.48g Urea. Add 0.7 ml deionized H_2O and vortex until the urea crystal dissolves completely. Final volume and concentration will be 1 ml of 8M urea. Dissolved urea can be stored at -20 ºC for a week.

HUMAN PEDF STANDARD PREPARATION

1. Rehydrate recombinant Human PEDF Standard (0.5µg) with 250µl **1x** Assay Diluent, vortex. Allow the solution to sit at least 10 minutes at room temperature (20-23°C) to completely dissolve in solution. Vortex and centrifuge before use. The concentration of this stock solution is 2000ng/ml.
2. Prepare Human PEDF standard solutions as follows:

Standard No.	Std. volume	Assay Buffer	Concentrations
Stock	Powder	250µl	2000ng/ml
#1	32µl of Stock	424µl	140ng/ml
#2	250µl of #1	250µl	70ng/ml
#3	250µl of #2	250µl	35ng/ml
#4	250µl of #3	250µl	17.5ng/ml
#5	250µl of #4	250µl	8.8ng/ml
#6	250µl of #5	250µl	4.4ng/ml
#7	250µl of #6	250µl	2.2ng/ml



PEDF (Human) ELISA PROTOCOL

1. Thoroughly read this protocol before performing an assay. Allow all reagents to come to room temperature (20-23°C) prior to the start of the assay.
2. Prepare the test samples by treating with urea as follows:
   (A)Pipetting 50µl of sample or specimens, and mix with 50µl of 8M Urea solution in an Eppendorf tube.
   (B)Incubate the tube at 4°C for 1 hour to denature the samples or specimens.
   (C)Dilute all the urea-treated samples at least 1:50 with Assay Diluent before adding to the microplate well.
   (D)All samples after dilution must be applied to the plate as soon as possible to minimize reassociation of the protein.
3. Remove Capture Antibody-Coated Plate from its zip-lock foil pouch. Remove unneeded strips from the plate frame, reseal them in the foil pouch, and return the foil pouch to 4°*C*.

4. Leave wells A-1 and A-2 empty as **Blank**.
5. Add 100µl of the prepared Human PEDF Standard solutions from **#7** to **#1** (reverse order of serial dilution) in duplicate to each well.
6. Add 100µl of Human PEDF Positive Control solution in duplicate.
7. Add 100µl diluted samples in duplicate into their designated wells.
8. Seal the immunoplate with Acetate Plate Sealer (APS). Incubate for 2 hours at 37°C on a plate shaker (300-400 rpm).
9. Before washing the plate, remove the plate sealer carefuly. Completely discard the liquid from wells. Wash each well with 300-350µl Wash Buffer four times. At the end of each the wash, discard the buffer, invert the plate, and tap on a clean absorbent towel.
10. Dilute Biotinylated anti-PEDF (Human) Detection Antibody to 1:100 as needed. Add 100µl diluted biotinylated anti-Human PEDF Detection Antibody into each well. Reseal the immunoplate with plate sealer and incubate for 1 hours at 37°C on a plate shaker (300-400rpm).
11. Wash 4 times with the **1x** Wash Buffer as described in step 9.
12. Add 100µl diluted SA-HRP solution into each well. Reseal the immunoplate with plate sealer and incubate the plate for 1 hour at 37°C on plate shaker (300-400rpm).
13. Wash 4 times with the **1x** Wash Buffer as described in step 9.
14. Add 100µl Substrate Solution (TMB) provided in this kit into each well. Reseal the plate with plate sealer to protect from light and incubate the plate for 30-40 minutes at room temperature (20-23°C) on a plate shaker (300-400 rpm). After incubation, blue coloring should be observed in the 70ng/ml standard wells.
15. Add 100µl Stop Solution (2N Hydrochloric Acid) into each well to stop the reaction. The color in the well should change from blue to yellow. If the color change does not appear to be uniform, gently tap the plate to ensure thorough mixing. Proceed to the next step within 20 minutes.
16. Read Absorbance O.D. at 450nm using a Microtiter Plate Reader.

ADDITIONAL RECOMMENDED PROCEDURAL NOTES:

- Reagents of different lot numbers shoud not be mixed.
- Recheck the reagent labels when loading the plate to ensure that everything is added correctly.
- Unused microplate strips should be placed in the foil pouch with a dessicant and stored at 4°C. Do not allow moisture to enter the wells.
- When handling the plate, avoid touching the bottom.
- Manual washing may cause high duplicate coefficient variations. To reduce this factor, liquid from the plate should be removed by inverting and blotting the plate on an absorbent material.
- If the room temperature is not within the suggested range (20-23°C), variations in results may occur.
- The same reservoir for the reagents may be reused if the reservoir is washed well with distilled water before each use.
- Each laboratory must determine the appropriate dilution factors for the samples to be measured to ensure that the samples are within the dynamic range of the standard curve.
- High levels of interfering proteins may cause variations within the sample results, therefore, it is imperative to select the appropriate sample preparation procedure to obtain the optimal results.
- Each time a new tip is used, make sure the tip is secure and free of air bubbles. For better intra-assay variation, aspirate and expel a reagent or sample back into the container a few times prior to loading.
- Avoid submerging the whole tip into reagents because droplets can accumulate at the end of the tip causing an excess of reagent to be loaded into the well. This can lead to poor results.
- For optimal results, an orbital plate shaker capable of 300-500 rpm is recommended for all incubations.
- Modification of the existing protocol (i.e. standard dilutions, pipetting technique, washing technique, incubation time or temperature, storage conditions, and kit expiration) may affect the sensitivity and specificity of the test.

SUMMARY OF ASSAY PROTOCOL

Add 100µl/well of Human PEDF Standard, Positive Control or Samples

▼

Incubate at 37°C for 2 hours

▼

Wash immunoplate 4 times with 350µl/well of **1x** Wash Buffer

▼

Add 100µl/well of Biotinylated Anti-Human PEDF Detection Antibody

▼

Incubate at room 37°C for 1 hour

▼

Wash immunoplate 4 times with 350µl/well of **1x** Wash Buffer

▼

Add 100µl/well of SA-HRP solution

▼

Incubate at 37°C for 1 hour

▼

Wash immunoplate 4 times with 350µl/well of **1x** Wash Buffer

▼

Add 100µl/well of Substrate Solution (TMB)

▼

Incubate at room temperature (20-23°C) for 30-40 minutes

▼

Terminate reaction with 100µl/well of 2N HCl

▼

Read absorbance O.D. at 450nm and calculate results

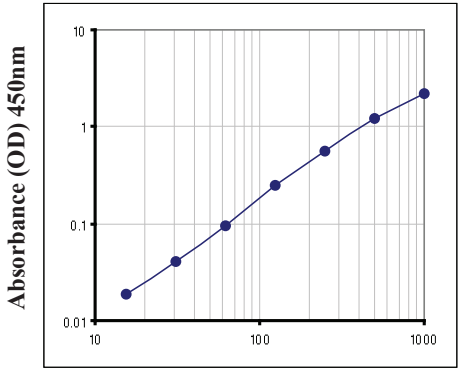
CALCULATION OF RESULTS

Plot the standard curve on log-log graph paper. Known concentration of Human PEDF Standard and its corresponding O.D. reading is plotted on the log scale (X-axis) and the log scale (Y-axis), respectively. The standard curve shows a correlated relationship between Human PEDF concentrations and the corresponding O.D. absorbance. As the standard concentration increases, the intensity of the yellow color, and in turn the O.D. absorbance, increases.

The concentration of Human PEDF within a sample is determined by plotting the sample's O.D. on the Y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line dropped from this point will intersect the X-axis at a coordinate corresponding to the Human PEDF concentration in the unknown sample.

Refer to QC Data sheet for acceptable values of the positive conrtol.

Human PEDF Standard Curve



STORAGE

1. Store the kit at 4°C upon receipt. The kit should be equilibrated to room temperature (20-23°C) before assay.
2. Store **1x** Assay Buffer at 4°C.
3. Remove any unneeded strips from Human PEDF antibody-Coated plate, reseal them in zip-lock foil and keep at 4°C.
4. Keep rehydrated solution of Human PEDF Standard, Biotinylated anti-Human PEDF Detection Antibody and SA-HRP at 4°C. Prepare only the required amount.

NOTE:

1. It is recommended that the solutions be used on the same day of rehydration.
2. After adding Stop Solution, read the plate within 20 minutes.

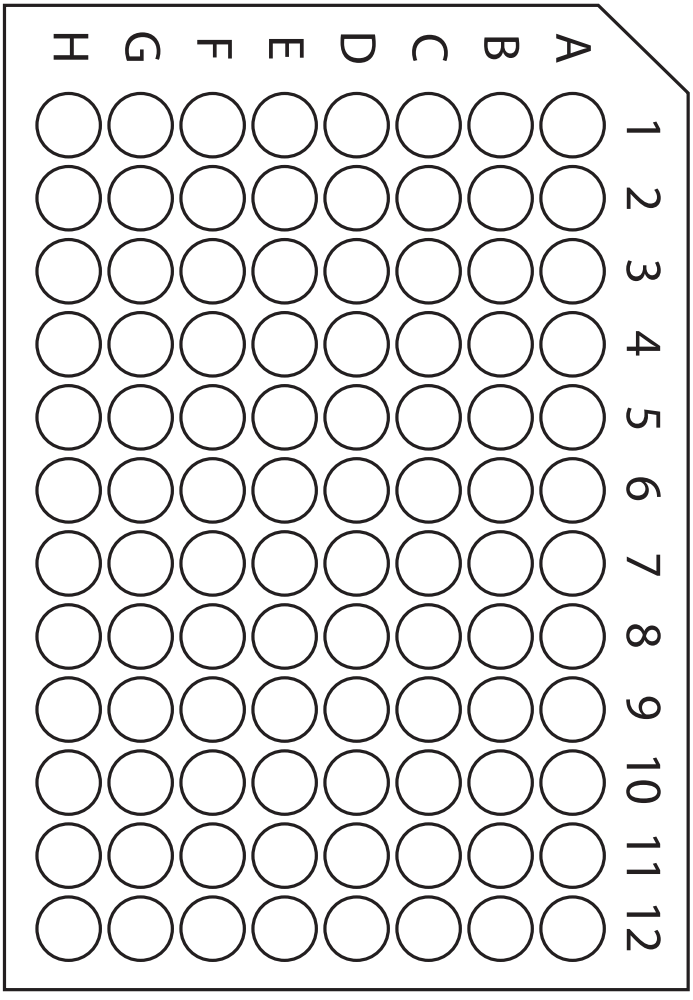
REFERENCES

1. Markeeva N, Lisovskiy I, Lyzogubov V, Usenko V, Soldatkina M, Merentsev S, Zaitsev S, Kondratskii Y, Tofan A, Osinskiy S, Pogrebnoy P. (2005) Expression of beta-defensin-2 in human gas tric tumors: a pilot study. R.E. Kavetsky Institute of Expre imental Pathology, Oncology and Radiology NAS of Ukraine, Kyiv, Ukraine. Exp Oncol.; 27(2):130-5.
2. Mineshiba J, Myokai F, Mineshiba F. Matsura K, Nishimura F, Takashiba S. (2005) Transcriptional regulation of beta-defensin-2 by lipopolysachharide in cultured human cervical carcinoma (HeLa) cells. FEMS Immunol Med Microbiol.: 45(1):37-44.
3. Schoder JM, Harder J. (1999) Human beta0defensin-2. Int. J Biochem Cell Biol. Department of Dermatology, University of Kiel, Germany; 31(6):645-51.

NOTES

NOTES

ASSAY DIAGRAM



USA

Phoenix Pharmaceuticals, Inc.

330 Beach Rd.
Burlingame, California 94010
Tel: 650-558-8898, 800-988-1205
Fax: 650-558-1686
Info@PhoenixPeptide.com
www.PhoenixPeptide.com

Europe

Phoenix Europe GmbH

Viktoriastrasse 3-5
D-76133 Karlsruhe
Germany
Tel: +49 (721)-12 08 15 0
Fax: +49 (721)-12 08 15 15
Germany@PhoenixPeptide.com