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ChemiKine™
Pigment Epithelium-Derived Factor (PEDF) Sandwich ELISA Kit

Cat. No. CYT420

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Pigment Epithelium-Derived Factor (PEDF) was first identified as a factor secreted by primary retinal pigment epithelial (RPE) cells that promotes the survival of neurons *in vitro* and *in vivo*. Molecular characterization of PEDF shows it to be a 50 kDa member of the serpin family of protease inhibitors[1]. A second mode of action of PEDF was identified with the demonstration that PEDF induces apoptosis of microvascular endothelial cells, a property that maintains the avascular nature of the retina[2]. As a number of ophthalmologic disorders are characterized by dysregulation of the retinal innervation and microvasculature, PEDF serves as an important regulator of human eye disease[3]. In addition, PEDF appears to have multifunctional antitumor activity in a neuroblastoma model, as Schwann cell-derived PEDF induces a differentiated, less malignant phenotype in neuroblastoma cells, promotes further growth and survival of Schwann cells, and inhibits angiogenesis[4].

***ChemiKine*™** Pigment Epithelium-Derived Factor (PEDF) kit is a sandwich enzyme immunoassay (EIA), which measures PEDF. The kit will measure PEDF of vitreous fluid or serum of human origin.

Test Principle

With the ***ChemiKine*™** PEDF assay system, a mouse monoclonal antibody generated against human PEDF is coated onto a microplate and is used to capture PEDF from a sample. A second, biotinylated PEDF-specific mouse monoclonal antibody detects the captured PEDF. Peroxidase-labeled streptavidin detects the biotinylated mouse monoclonal antibody. After addition of the substrate and stop solution the amount of PEDF is determined. The standard curve demonstrates a direct relationship between Optical Density (OD) and PEDF concentration: i.e., the higher the OD the higher the PEDF concentration in the sample.

Application

***ChemiKine*™** PEDF kit is designed to measure the amount of PEDF in cell culture supernatants, tissue homogenates and biological fluid (vitreous, serum, plasma, and serum-free) samples of human origin. There are enough reagents included in this kit for one 96-well immunoassay plate. Running duplicate wells for samples and standards is recommended.

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Analytical Sensitivity and Detection Limits

Sensitivity:	0.9 ng/mL
Range of Detection:	0.9 ng/mL to 62.5 ng/mL
Intra-assay Variation:	±5.3% (7.5 ng/mL)
Inter-assay Variation:	±16.0% (7.5 ng/mL)

Kit Materials

1. PEDF ELISA Plate: (Part No. 61597) One 96-Well Immunoplate Pre-coated with Mouse anti-Human PEDF Monoclonal Antibody, sealed in a foil pouch.
2. Wash Buffer Concentrate: (Part No. 60245) One 100 mL (10X) bottle of Concentrate.
3. Assay Diluent: (Part No. 60240) One 60 mL bottle (Ready to Use).
4. PEDF Standard (Human): (Part No. 61600) One vial containing 250 ng recombinant full length human PEDF (Lyophilized).
5. Biotinylated Mouse anti-Human PEDF Monoclonal Antibody: (Part No. 61598) One 50 µL vial.
6. Streptavidin, HRP conjugated: (Part No. 61599) One 50 µL vial.
7. TMB/E Solution: (Part No. 60096) One 10 mL bottle of a Ready to Use solution of 3,3',5,5'-tetramethylbenzidine in a proprietary buffer with enhancer.
8. Stop Solution (Part No. 60193): One 12 mL bottle of an HCl solution (Ready to Use).

Materials Not Supplied

1. Multi-channel or repeating pipettes
2. 37ºC incubator
3. Plate sealers
4. Pipettors & tips capable of accurately measuring 10-1000 µL
5. Graduated serological pipettes
6. 96-well microplate reader with 450 nm filter
7. Graph paper for manual plotting of data
8. Polypropylene test tubes for standard and sample dilutions
9. Mechanical vortex
10. One 1 or 2 liter container
11. Urea

Precautions

- Wash Buffer and Assay Diluent contain thimerosal. Thimerosal is highly toxic by inhalation, contact with skin or if swallowed. Thimerosal is a possible mutagen and should be handled accordingly.
- The instructions provided have been designed to optimize the kit's performance. Deviation from the instructions may result in suboptimal performance of the kit and the failure to produce accurate data.

Technical Hints

- Manual Plate Washing: Vigorous washing and complete removal of all liquid by aspiration at the end of each washing step is very important to obtain low background values.
- **Recommended Method for Plate Washing:**
    1. Remove existing fluid from each well by flicking the plate over a sink. Subsequently blot the plate on clean paper towels.
    2. Forcefully pipet 250 µL of diluted Wash Buffer into each well with a multi-channel pipet.
    3. Remove fluid from each well by flicking the plate over a sink. Subsequently blot the plate on clean paper towels.
    4. Repeat washing and flicking 4 times.

Preparation of Reagents

1. **Wash Buffer**

   Add the entire contents of the 10X Wash Buffer Concentrate to appropriate container, QS to 1 liter with deionized water. Stir to homogeneity.

2. **PEDF Standard**

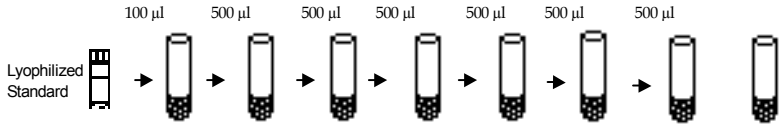
   ***Note**: When opening lyophilized Standard, remove rubber stopper gently as the lyophilizate may have become dislodged during shipping.*

   Reconstitute the standard vial with 500 µL of water to give a relative PEDF concentration of 500 ng/mL. Use within 1 h and store the unused PEDF stock solution at -20°C in its original vial up to 30 days. This stock material is then used to generate a standard curve. Use the Assay Diluent to make the dilutions. A suggested dilution scheme is as follows:

   a) Label 7 test tubes #1-7 and "0 dose". Add 700 µL of the Assay Diluent to Standard tube #1. Add 500 µL of the Assay Diluent to Standard tubes #2-7 and the "0 dose".

   b) Add 100 µL of the stock Standard solution to tube #1 and mix by inverting several times. This is Standard tube #1 with a concentration of 62.5 ng/mL.

   c) Standards #2-7 are then prepared by performing a 1:2 dilution of the preceding standard. Refer to Fig. 1. For example, to make Standard #2, remove 500 µL of Standard #1 and add it to tube #2, mix thoroughly by inversion, and so on. Do not add any PEDF Standard to the "0 Dose" Standard tube.



Standard Number:	#1	#2	#3	#4	#5	#6	#7	0 dose
Initial Volume (µL):	700	500	500	500	500	500	500	500
Concentration (ng/mL):	62.5	31.3	15.6	7.8	3.9	1.95	0.98	0.0

Figure 1: Serial Dilution of PEDF Standard

***Note:** The PEDF Standard curve can be set up with a different serial dilution scheme by making appropriate adjustments to the dilution pattern.*

3. **Biotinylated Mouse Anti-Human PEDF Monoclonal Antibody**

   Immediately before use, dilute the anti-PEDF monoclonal 1:500 with Assay Diluent. Do not store diluted antibody

4. **Streptavidin, HRP Conjugated**

   Immediately before use dilute the streptavidin, HRP conjugated 1:1,000 with Assay Diluent. Do not store diluted solution.

Preparation of Samples

It is recommended that each sample be tested in duplicate. The samples should be diluted with Assay Diluent 1:2 and further diluted by a two-fold serial dilution and run down a column of the plate. Alternatively, samples can be screened at a single concentration (in triplicate) and all positive samples retested to determine the exact PEDF concentration.

Tissue samples should be rapidly excised, weighed and snap frozen in liquid nitrogen prior to storage at -70°C. Within two weeks of freezing, tissue samples should be homogenized in an ice-cold homogenization buffer.

Although the user will need to optimize the extraction buffer based on the nature of the tissue sample and the degree of extraction desired, a suggested extraction buffer is 100mM Tris/HCl, pH 7.0, containing 2% bovine serum albumin (BSA), 1M NaCl, 4mM EDTA.Na_2, 2% Triton X-100, and the protease inhibitors (Sigma) 5 µg/mL aprotinin, 0.5 µg/mL antipain, 157 µg/mL benzamidine, 0.1 µg/mL pepstatin A and 17 µg/mL phenylmethyl-sulphonyl fluoride.

Since sodium azide (NaN_3) inhibits horseradish peroxidase activity, avoid use of any buffer containing sodium azide to prepare or dilute samples. Homogenates should be prepared in approximately 20 to 100 volumes of the homogenization buffer to tissue wet weight, but the most appropriate ratio needs to be determined by the user for each tissue. The homogenates are centrifuged at 14,000xg for 30 minutes. The resulting supernatants should be used for the PEDF assay.

A number of studies have shown that in vitreous PEDF can be complexed, most prevalently to fibrillar collagens and glycosaminoglycans[5]. The composition of the extraction buffer will determine the extent to which PEDF is released from its binding proteins. In addition, PEDF belongs to the serpin family of protease inhibitors, members of which undergo dramatic changes in structure upon binding to target proteases[6]. Although structural and biochemical studies indicate that PEDF does not change conformation upon cleavage by proteases[7], the possibility exists that PEDF undergoes conformational changes under other circumstances. To minimize potential differences in conformation between test samples that could result in changes in immunoreactivity of PEDF, test samples should be treated with identical extraction and dilution buffers, and stored under identical conditions. ***PEDF may be present in samples in different formats bound or unbound on different conformation not recognized by this assay system. In order to measure total PEDF or to compare bound with unbound PEDF, it may be necessary to treat the sample with urea (step 2 of Assay Instructions) before beginning the assay.***

Storage

Maintain the unopened kit at 2-8°C until expiration date indicated on the label. After opening the kit maintain each component at 2-8°C until expiration date indicated on the label. After reconstitution, maintain the PEDF Standard at -20ºC for up to 30 days. The standard may be frozen and thawed three times without loss of immunoreactivity. Do not store reconstituted standard at 4ºC, as loss of immunoreactivity will result.

Assay Instructions

1. Place the desired number of *ChemiKine* Pigment Epithelium-Derived Factor (PEDF) strips in the strip well plate holder.
2. If measurement of total PEDF is desired, treat samples with urea as follows:
   A. Add urea to the sample to a final concentration of 8M and incubate on ice for 1 hour.
   B. Dilute urea-treated samples at least 1:100 in assay diluent (Part No. 60240) or PBS, Tween with 1% BSA to minimize the effect of urea on binding of the antigen to the antibodies.
   C. Samples must be applied to the plate immediately after dilution to minimize reassociation of the antigen to binding proteins.
3. Add 100 µL of Standards 0 through 7 or samples to wells. It is recommended that standards and samples be run in duplicate.

   Note: *A standard curve must be run at each setting.*
4. Seal the plate with a plate sealer. Incubate the plate for 1 hour at 37ºC.
5. **IMPORTANT WASH STEP:**

   Gently remove the plate sealer and wash the plate at least 4 times. A thorough washing of the plate is extremely important to reduce background. We recommend using a multi-channel pipette to fill each well with 250 µL of diluted Wash Buffer. Fluid removal from the wells is best accomplished by inverting the plate over a sink, flicking the fluid out of the wells, and blotting the plate on clean paper towels. Using the multichannel pipet add 250 µL of Wash Buffer to each well; flick and blot the plate. Repeat this procedure for a total of 4 times.

   For users of automatic plate washers: It is important to ensure that the wash apparatus is properly maintained and operating correctly. Tubing and tips can easily become clogged, leading to incomplete washing and inadequate aspiration of wells. The result may be poor precision and an unsuitable standard curve. For best results, we recommend at least 4 wash cycles.

6. Add 100 μL of the diluted Biotinylated Mouse Anti-Human PEDF monoclonal antibody (see reagent preparation section) to each well. Cover the plate and incubate at 37ºC for 1 hour. Wash as described in Step 4.

7. Add 100 μL of the diluted streptavidin peroxidase conjugate (see reagent preparation section) to each well. Cover the plate and incubate at 37ºC for 1 hour. Wash as described in Step 4.

8. Warm TMB/E to room temperature. Add 100 μL of TMB/E Substrate to each well. Incubate at room temperature for 5-10 minutes. (The 62.5 ng/mL standard should achieve a deep blue color). Stop the reaction by adding 100 μL of Stop Solution to each well. The blue color will change to yellow. Immediately read the plate at 450 nm (color will fade over time).

   CAUTION: *Bubbles in the wells will cause inaccurate readings. Ensure that all bubbles are removed prior to taking the absorbance reading.*

Calculation of Results

Manual Plotting: Plot the standard curve on graph paper. Known concentrations of PEDF are plotted on the X-axis and the corresponding OD on the Y-axis. The standard curve should result in a graph that shows a direct relationship between PEDF concentrations and the corresponding ODs (absorbances). In other words, the greater the concentration of PEDF in the sample, the higher the OD. The concentration of PEDF in unknown samples may be determined by plotting the sample OD on the Y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line dropped from this point intersects the X-axis at the concentration of PEDF in the unknown sample.

Plate Reader/PC Interface: An alternative approach is to enter the data into a computer program curve fitting software. A good fit can be obtained with a linear regression analysis. Some data points at the top or bottom of the range tested may need to be dropped to get a good fit. Currently existing spreadsheet software can perform such plotting.

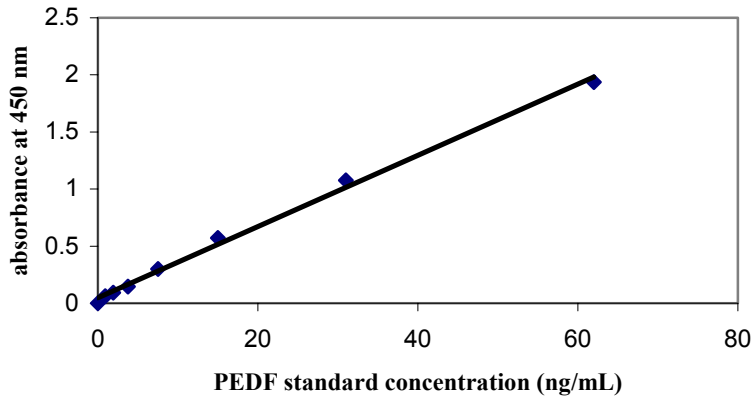


Figure 1. Representative standard curve generated with *ChemiKine*™ Pigment Epithelium-Derived Factor (PEDF) sandwich ELISA kit.

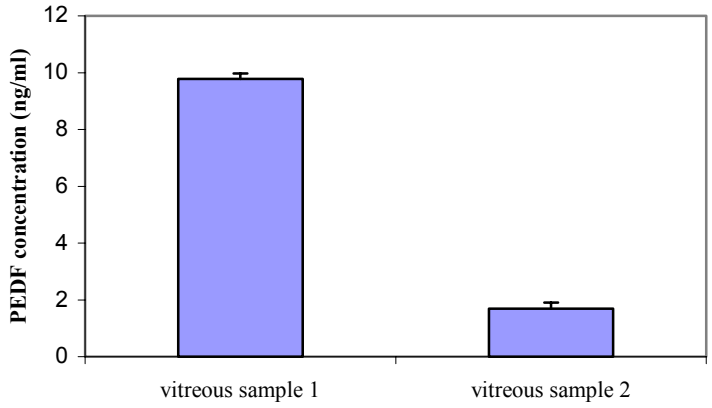


Figure 2. Determination of PEDF concentrations in human vitreous fluid with *ChemiKine*™ Pigment Epithelium-Derived Factor (PEDF) sandwich ELISA kit.

Reference:

1. Tombran-Tink, J. et al. (1991) PEDF: a pigment epithelium-derived factor with potent neuronal differentiative activity. Exp. Eye Res. 53: 411-414.
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3. Ogata, N. et al. (2001) Pigment epithelium-derived factor in the vitreous is low in diabetic retinopathy and high in rhegmatogenous retinal detachment. Am. J. Ophthalmol. 132: 378-382.
4. Crawford, S.E. et al. (2001) Pigment epithelium-derived factor (PEDF) in neuroblastoma: a multifunctional mediator of Schwann cell antitumor activity. J. Cell Sci. 114: 4421-4428.
5. Meyer, C. et al. (2002) Mapping the type I collagen-binding site on pigment epithelium-derived factor. J. Biol. Chem. 277: 45400-45407.
6. Silverman, G.A., et al. (2001) The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. J. Biol. Chem. 276: 33293-33296.
7. Simonovic, M., et al. (2001) Crystal structure of human PEDF, a potent anti-angiogenic and neurite growth-promoting factor. Proc. Natl. Acad. Sci. USA 98: 11131-11135.

Warranty

These products are warranted to perform as described in their labeling and in ***CHEMICON***® literature when used in accordance with their instructions. THERE ARE NO WARRANTIES WHICH EXTEND BEYOND THIS EXPRESSED WARRANTY AND CHEMICON® DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. ***CHEMICON's***® sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of ***CHEMICON***®, to repair or replace the products. In no event shall ***CHEMICON***® be liable for any proximate, incidental or consequential damages in connection with the products.

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