



Human PEDF ELISA Assay

Catalog# PED613-Human

Introduction

Principle of the Assay

Microwells are coated with a polyclonal antibody specific for full-length recombinant human PEDF antigen. The PEDF antigen present in the specimen is specifically captured onto the immobilized antibody during specimen incubation. The captured antigen is then detected with a high-titer polyclonal anti-PEDF antibody conjugated to biotin. Following subsequent incubation with streptavidin peroxidase, the bound enzyme reacts with a substrate to yield a colored product. The resultant optical density is proportional to the amount of PEDF antigen present in the specimen.

Kit Presentation

Materials Supplied

The reagents supplied in this pack are for research use only.

1	huPEDF Antibody Coated Microplate	1 plate (96 wells)
2	huPEDF Antigen Standard, rubber-stoppered crimp-cap vial; contains PEDF antigen in a phosphate buffer diluent	14.5 ng
3	PEDF Detector Antibody (100X), green-cap tube; contains biotin-labeled polyclonal antibody to PEDF in a proprietary antibody stabilizing solution	150 µL
4	Streptavidin-Peroxidase (100X), orange or yellow cap tube; contains streptavidin conjugated to peroxidase, PBS, and Tween®	150 µL
5	Assay Diluent (1X); contains PBS and BSA	75 mL
6	8M Urea Lysing Buffer (red-cap tube); contains 0.48g urea, will yield 8M urea upon addition of 0.7 mL deionized water	0.48 g
7	Plate Wash Buffer (20X); contains Tris-Buffered Saline and Tween 20®.	50 mL
8	TMB Substrate; contains tetramethylbenzidine (TMB), hydrogen peroxide, and dimethyl sulfoxide	12 mL
9	Stop Solution	12 mL
10	Plate Sealers	3 Sheets

Materials Required but Not Supplied

- > Adjustable micropipettes (single and multichannel)
- > Test tubes and rack for preparing specimen and control dilutions
- > Graduated cylinders and assorted beakers
- > Incubator for 37°C ± 1°C
- > Microplate reader capable of reading at 450nm and 650nm
- > Timer
- > Distilled or deionized water

Storage and Stability

All reagents should be stored at 2-8°C and should not be used beyond the expiration date on the label. Once opened, microtitration strips may be stored at 2-8°C until the expiration date on the label, provided that desiccated conditions are maintained. Unused strips should be returned to their original foil pouch along with the sachet of desiccant. Secure open foil pouch using zip top before storage.

The prepared 1X Wash Buffer should not be stored for longer than 1 week at 2-8°C. It is recommended that Wash Buffer be freshly diluted before each assay. If the prepared 1X becomes visibly cloudy or develops precipitate during the 1-week storage, do not use it.

Indications of Deterioration

The Human PEDF ELISA Assay may be considered to have deteriorated if:

1. The kit fails to meet the required criteria for a valid test (see Interpretation of Results).
2. Reagents become visibly cloudy or develop precipitate. *Note: 20X Concentrated Wash Buffer, when cold, normally develops crystalline precipitates, which re-dissolve on heating at 37°C.*
3. The Substrate Solution turns dark blue. This is likely to be caused by chemical contamination of the Substrate Solution.

Warnings and Precautions

1. The reagents supplied in this kit are for **research use only**.
2. Prior to performing the assay, carefully read all instructions.
3. Use universal precautions when handling kit components and test specimens.
4. To avoid cross-contamination, use separate pipet tips for each specimen.
5. When testing potentially infectious human specimens, adhere to all applicable local, state and federal regulations regarding the disposal of biohazardous materials.
6. Stop Solution contains hydrochloric acid which may cause severe burns. In case of contact with eyes or skin, rinse affected area immediately with water and seek medical assistance. Wear protective clothing and eyewear.

Method of Use

Plate Washing Procedure

Manual Washing:

Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a multichannel pipet, add at least 200 µL of 1X Wash Buffer to each well, and then aspirate contents of the plate into a sink or proper waste container. Alternatively, using a squirt bottle, fill each well completely with Wash Buffer and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a total of five (5) washes. After the final wash, invert plate, and blot dry by striking plate onto absorbent paper or paper towels until no moisture appears. **NOTE:** Hold the sides of the plate frame firmly when washing the plate to ensure that all strips remain securely in frame.

Automated Washing:

Aspirate all wells, then wash plates five (5) times using 1X Wash Buffer. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by striking plate onto absorbent paper or paper towels until no moisture appears.

Preparation for the Assay

PEDF Antigen Standard

Add 145 µL of Assay Diluent to the lyophilized PEDF Antigen Standard (rubber-stoppered crimp-cap vial); mix periodically for 5 minutes with gentle agitation to ensure the PEDF Antigen Standard is dissolved completely. The final PEDF concentration in the PEDF Antigen Standard stock solution is 100 ng/mL. Prepare a series of six standards from the PEDF Antigen Standard stock solution. Use the dilution scheme provided in Table 1 below. Any diluted PEDF Antigen Standard remaining after the completion of the assay should be discarded appropriately; do not save diluted PEDF Antigen Standard.

Standard #	PEDF Antigen Standard (µL)	Assay Diluent (µL)	Concentration of PEDF (ng/ml)
1	10 µL of stock sol'n	990 µL	1.00
2	500 µL of #1	500 µL	0.50
3	500 µL of #2	500 µL	0.25
4	500 µL of #3	500 µL	0.13
5	500 µL of #4	500 µL	0.06
6	500 µL of #5	500 µL	0.03
7	0	500 µL	0

Table 1: Preparation of Antigen Standard

PEDF Detector Antibody Working Solution

Dilute 120 µL of 100X PEDF Detector Antibody (green-cap tube) to 12 mL final volume using Assay Diluent; mix gently prior to use. Use the diluted PEDF Detector Antibody Working Solution within 15 minutes of preparation; assay sensitivity decreases if the PEDF Detector Antibody is not freshly diluted. Any diluted PEDF Detector Antibody Working Solution remaining after completion of the assay must be discarded.

Streptavidin-Peroxidase Working Solution

Dilute 120 µL of 100X Streptavidin-Peroxidase (orange or yellow cap tube) to 12 mL final volume using Assay Diluent; mix gently prior to use. Any diluted Streptavidin-Peroxidase Working Solution remaining after completion of the assay must be discarded.

To prepare smaller volumes of the PEDF Detector Antibody and Streptavidin-Peroxidase Working Solutions for partial-plate assays, use the dilution schemes provided in Table 2.

Number of Strips Used	PEDF Detector Antibody Working Solution		Streptavidin-Peroxidase Working Solution	
	PEDF Detector Antibody (100X) (µL)	Assay Diluent (mL)	Streptavidin-Peroxidase (100X) (µL)	Assay Diluent (mL)
3	40	3.96	40	3.96
6	70	6.93	70	6.93
9	100	9.90	100	9.90
12	120	11.88	120	11.88

Table 2: Preparation of PEDF Detector Antibody and Streptavidin-Peroxidase Working Solutions

8M Urea

Add 0.7 mL deionized water to the red-cap tube containing 0.48 g urea. Vortex vigorously for 30-60 sec until urea completely dissolved. Final volume and concentration will be 1.0 mL of 8M urea. Dissolved urea can be stored at -20°C for 6 months.

Plate Wash Buffer

Dilute 20X Plate Wash Buffer 1:20 (i.e., 50 mL of 20X Wash Buffer diluted to 1 liter final volume) with distilled or deionized water prior to use. Store 1X Plate Wash Buffer at 2-8°C for up to one (1) week.

Assay Procedure

Allow all reagents to reach room temperature before use. Label test tubes to be used for the preparation of standards and specimens. Label each strip on its end tab to identify the strips should they become detached from the plate frame during the assay. If the entire 96 well plate is not used, remove surplus strips from the plate frame. Place surplus strips and desiccant into the Resealable Foil Bag, seal and store at 2° - 8°C.

Assay Protocol

- OPTIONAL:** (if PEDF is complexed with cellular proteins): Treat specimens in an Eppendorf tube, or equivalent, by pipetting 50 µL Lysing Buffer (8M Urea) into 50 µL specimen and mixing well. Let samples lyse for 15-30 minutes at 4°C with periodic mixing (every 5-10 min). Dilute all urea-treated samples at least 1:50 before adding to the microplate.
- Pipet 100 µL of standards #1-7 into duplicate wells. Leave one or two wells of the microtiter plate empty during the assay. These wells will be used for a substrate blank.
- Pipet 100 µL of each specimen, as prepared in Step 1, into duplicate wells. **NOTE:** Urea-treated samples must be diluted at least 1:50 before adding to the microplate well.
- Cover microplate with a plate sealer and incubate for 1 hour at 37°C ± 1°C
- Aspirate samples and wash each well of the microplate or the selected number of strips 5 times with ≥200 µL of 1X Plate Wash Buffer and aspirate. Thoroughly blot by striking inverted microplate or strips on a pad of absorbent towels. Continue striking until no droplets remain in the wells.
- Pipet 100 µL of reconstituted PEDF Detector Antibody into each well, except the substrate blank.
- Cover the microplate with a sealer and incubate for 1 hour at 37°C±1°C.
- Aspirate and wash plate 5 times as described in Step 5.
- Pipet 100 µL of the Streptavidin Peroxidase Working Solution into each well except the substrate blank.
- Cover the microplate with a sealer and incubate for 30 minutes at 37°C±1°C.
- Aspirate and wash plate 5 times as described in Step 5. **IMPORTANT:** Thorough washing of the wells at this point is critical for attaining low background values in the assay.
- Pipet 100 µL of TMB Substrate into all wells and incubate uncovered for 20-30 minutes at room temperature (18°-25°C). **NOTE:** More or less incubation time might be required for color development depending on the ambient temperature of your test facility. A blue color will develop in wells containing PEDF antigen.
- Stop the reaction by pipetting 100 µL of Stop Solution into each well. A color change from blue to yellow will result.
- Read the optical density of each well at 450 nm using a microplate reader (650 nm is the appropriate reference wavelength for TMB substrate reactions); the color is stable for approximately 2 hours.

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Interpretation of Results

Test Validity

Determine the mean optical density values for each standard and specimen.

For the test to be valid, it must meet the following criteria:

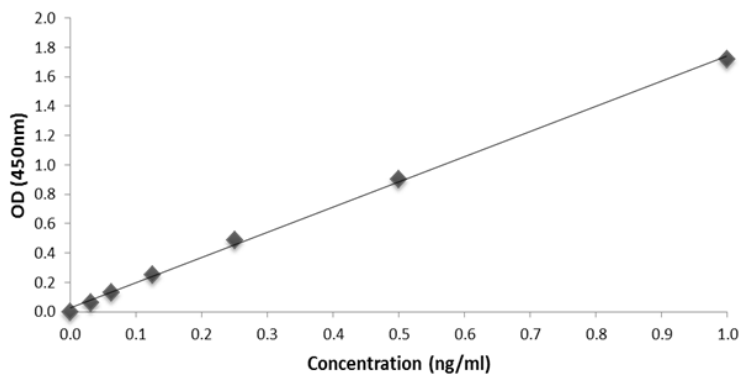
- The mean optical density of the 0 ng/mL standard and the substrate blank must be less than 0.300. Subtract the substrate blank from the mean optical density of 0 ng/ml
- The mean optical density of the 0.5 ng/mL PEDF standard must be greater than or equal to 0.500.

Quantifying Levels of PEDF Antigen

Using linear graph paper, plot the concentration of PEDF Antigen Standard (ng/ml) on the X-axis versus the mean optical densities for each standard on the Y-axis. Then determine the concentration of PEDF antigen in specimens by interpolation from the standard curve. Alternatively, the level of PEDF may be calculated by computer using a point-to-point algorithm. Be sure to correct for all dilutions. Typical results obtained with the PEDF ELISA are shown below:

Concentration (ng/mL)	Mean (Adjusted) (OD)	Standard Deviation	%CV
1.00	1.721	0.096	5.58
0.50	0.905	0.032	3.54
0.25	0.489	0.011	2.25
0.13	0.253	0.006	2.37
0.06	0.132	0.008	6.06
0.03	0.061	0.009	14.75
0	0	0.006	N/A

Table 3: Typical PEDF ELISA Results



Graph 1: Typical PEDF Standard Curve

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