Human PEDF ELISA Kit Manual

Cat. No.: PED613 – Human Lot No.: 201691

IMPORTANT!

The protocol has changed. Please discard the old version of this manual and use the instructions provided within this manual version.

PRECAUTIONS

FOR RESEARCH USE ONLY. NOT FOR IN VITRO DIAGNOSTIC USE

- Prior to performing the assay, carefully read all instructions.
- Use universal precautions when handling kit components and test specimens.*
- To avoid cross-contamination, use separate pipet tips for each specimen.
- When testing potentially infectious human specimens, adhere to all applicable local, state and federal regulations regarding the disposal of biohazardous materials.
- 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.

PRINCIPLE OF THE TEST

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.

REAGENTS

Materials Supplied:

- huPEDF Antibody Coated Microplate for 96 determinations
- huPEDF Antigen Standard (14.5 ng), rubber-stoppered crimp-cap vial; contains PEDF antigen in a phosphate buffer diluent
- PEDF Detector Antibody (100X), 150 µL, green-cap tube; contains biotin-labeled polyclonal antibody to PEDF in a proprietary antibody stabilizing solution

- Streptavidin-Peroxidase (100X), 150 µL, yellow-cap tube; contains streptavidin conjugated to peroxidase, PBS, and Tween®
- Assay Diluent (1X), 75 mL; contains PBS and BSA
- 8M Urea Lysing Buffer (red-cap tube); contains 0.48g urea, will yield 8M urea upon addition of 0.7 mL deionized water.
- Plate Wash Buffer (20X), 50 mL; contains Tris-Buffered Saline and Tween 20®.
- TMB Substrate, 12 mL; contains tetramethylbenzidine (TMB), hydrogen peroxide, and dimethyl sulfoxide
- Stop Solution, 12 mL
- 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 or reading at 450nm and 650nm)
- Timer
- Distilled or deionized water

RECOMMENDATIONS

Storage:

- Store all kit reagents at 2°-8°C. When stored properly, the kit is stable until the date indicated on the box label.

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 assure that all strips remain securely in frame.

- Automated Washing:
  Aspirate all wells, and 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 OF REAGENTS

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 assure 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 Number	PEDF Antigen Standard (µL)	Assay Diluent (µL)	Concentration of PEDF (ng/mL)
#1	10 µL of stock soln.	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 PEDF 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 (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 -20C 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 room temperature for up to one (1) week.

TEST 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.

Step 1: **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 4ºC with periodic mixing (every 5-10 min). Dilute all urea-treated samples at least 1:50 before adding to the microplate.

Step 2: 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.

Step 3: 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.

Step 4: Cover microplate with a plate sealer and incubate for 1 hour at 37°C ± 1°C

Step 5: 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.

Step 6: Pipet 100 µL of reconstituted PEDF Detector Antibody into each well, except the substrate blank.

Step 7: Cover the microplate with a sealer and incubate for 1 hour at 37°C±1°C.

Step 8: Aspirate and wash plate 5 times as described in Step 5.

Step 9: Pipet 100 µL of the Streptavidin Peroxidase Working Solution into each well except the substrate blank.

Step 10: Cover the microplate with a sealer and incubate for 30 minutes at 37°C±1°C.

Step 11: 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.

Step 12: 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.

Step 13: Stop the reaction by pipetting 100 µl of Stop Solution into each well. A color change from blue to yellow will result.

Step 14: 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.

CALCULATIONS

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 0.200.
- The mean optical density of the 0.5 ng/mL PEDF standard must be greater than or equal to 0.500.

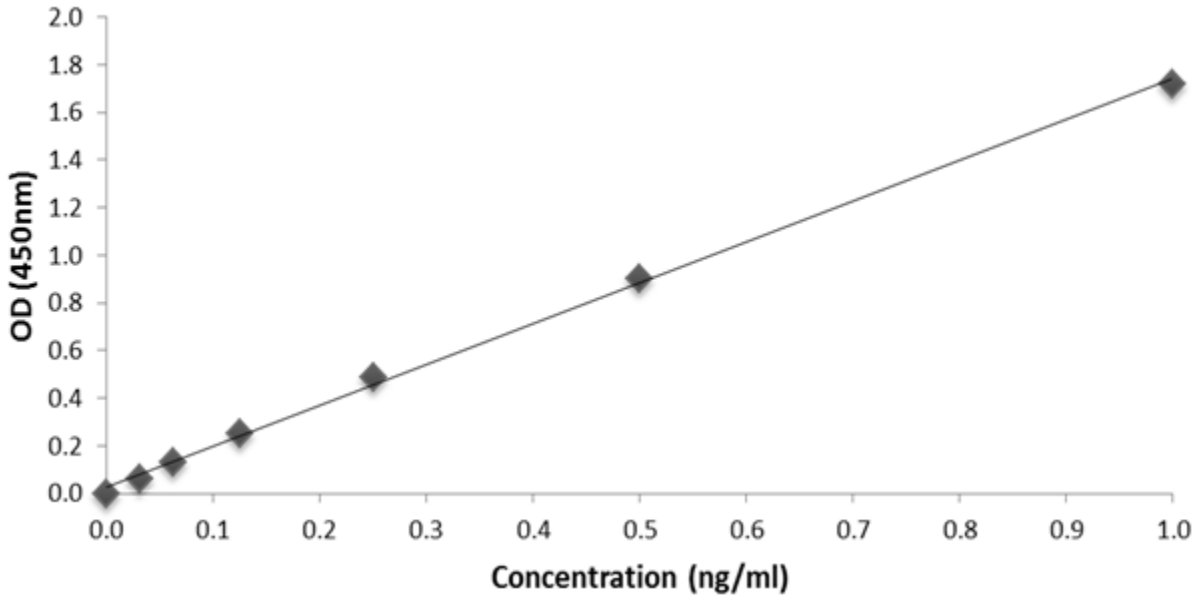
To Quantify Levels of PEDF Antigen:

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 ELISA quant™ 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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