Product Manual

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Protectin D1 (PD1) ELISA Kit

Catalog No.: abx257910

Size: 96T

Range: 0.156 ng/ml - 10 ng/ml

Sensitivity: 0.094 ng/ml

Storage: Store at 4°C for up to 6 months.

Application: For quantitative detection of PD1 in Serum, Plasma and other biological fluids.

Introduction: Protectin D1 also known as neuroprotectin D1 (when it acts in the nervous system) and abbreviated most commonly as PD1 or NPD1 is a member of the class of specialized proresolving mediators. Like other members of this class of polyunsaturated fatty acid metabolites, it possesses strong anti-inflammatory, anti-apoptotic and neuroprotective activity. Specifically, PD1 is an endogenous stereoselective lipid mediator classified as an autocoid protectin. Autacoids are enzymatically derived chemical mediators with distinct biological activities and molecular structures. Protectins are signaling molecules that are produced enzymatically from unsaturated fatty acids. Their molecular structure is characterized by the presence of a conjugated system of double bonds. PD1, like other protectins, is produced by the oxygenation of the ω-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) and it is found in many tissues, such as the retina, the lungs and the nervous system.

Principle of the Assay

This kit is based on a competitive enzyme-linked immuno-sorbent assay technology. PD1 is pre-coated onto a 96-well plate. The standards, samples and a biotin conjugated antibody specific to PD1 are added to the wells and incubated. After washing away the unbound conjugates, Streptavidin-HRP is added to each microplate well and incubated. After TMB substrate solution is added only wells that contain PD1 will produce a blue colour product that changes into yellow after adding stop solution. The intensity of the color yellow is inverse proportional to the PD1 amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of PD1 can be calculated.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standard: 2 tubes
3. Sample/Standard Diluent Buffer: 20 ml
4. Biotin conjugated antibody (Dilution 1:100): 60 µl
5. Antibody diluent buffer: 10 ml
6. Streptavidin-HRP Conjugate (Dilution 1:100): 120 µl
7. HRP Diluent Buffer: 10 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 30 ml

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.

» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

Sample dilution guideline:

Estimate the concentration of the target in the sample and select the correct dilution factor to make the diluted target concentration fall near the middle of the kit's range. Generally, for high concentration (100 ng/ml - 1000 ng/ml), dilute 1:100, for medium concentration (10 ng/ml - 100 ng/ml), dilute 1:10 and for low concentration (0.156 ng/ml - 10 ng/ml), dilute 1:2. Very low concentrations (≤ 0.156 ng/ml) do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

2. Wash buffer

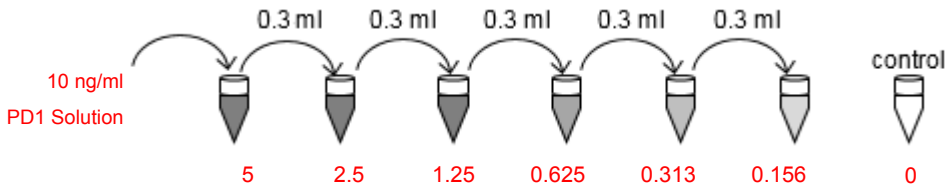
Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard

Preparation of the PD1 standard: standard solution should be prepared no more than 15 min prior to the experiment. Centrifuge at 10,000×g for 1 minute as the powder may drop off from the cap when opening if you do not spin down. **(Note: Do not dilute the standard directly in the plate).**

a.) 10 ng/ml standard solution. Add 1 ml of Sample/Standard diluent buffer into one Standard tube. Allow the reconstituted standard to sit for 15 minutes with gentle agitation prior to carrying out the serial dilutions; avoiding foaming or bubbles.

b.) 5 ng/ml → 0.15625 ng/ml standard solutions: Label 6 tubes with 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.3125 ng/ml and 0.15625 ng/ml. Aliquot 0.3 ml of the Sample / Standard diluent buffer into each tube. Add 0.3 ml of the above 10 ng/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard. Please use the diluted Standards for a single assay procedure and discard after use.

4. Preparation of Biotin conjugated antibody working solution: prepare no more than 1 hour before the experiment.
a.) Calculate the total volume of the working solution: 0.05 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the Biotin conjugated antibody with Antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of Antibody diluent buffer.

5. Preparation of Streptavidin-HRP working solution: prepare no more than 30 min before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the Streptavidin-HRP Conjugate with HRP diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Streptavidin-HRP Conjugate into 99 µl of HRP diluent buffer.

B. Assay Procedure
Equilibrate the TMB substrate at 37°C for 30 minutes prior to use. It is recommended to plot a standard curve for each test.

1. **Wash the plate two times before adding standard, samples and buffers.** Any strips that are not being used should be kept dry and stored at 4°C. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 50 µl of the prepared standards solutions into the standard wells.
3. Add 50 µl of Sample / Standard diluent buffer into the control (zero) well.
4. Add 50 µl of appropriately diluted sample into test sample wells.
5. Immediately add 50 µl of Biotin conjugated antibody working solution into each well. (Please add the solution at the bottom of each well without touching the side wall).
6. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 45 minutes.
7. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash buffer (350µL) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
8. Add 100 µl of Streptavidin-HRP working solution into each well, cover the plate with a new sealer and incubate at 37°C for 30 minutes.
9. Remove the cover and wash the plate 5 times with Wash Buffer as explained in step 7.
10. Add 90 µl of TMB substrate into each well. Cover the plate and incubate at 37°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.

11. Add 50 µl of Stop solution into each well (including the blank well). There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.

12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

This assay is competitive, therefore there is an inverse correlation between PD1 concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and absorbance measured (x-axis). Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1.Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.

2.Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard solutions within 15 min of starting the experiment. Please use the diluted Standard for a single assay procedure and discard after use.

3.It is recommended to assay all standards, controls and sample in duplicate. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.

4.Ensure plates are properly sealed or covered during incubation steps.

5.Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.

6.To avoid cross contamination do not reuse pipette tips and tubes.

7.Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.

8.The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of PD1 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of PD1 were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<8%

Inter-Assay: CV<10%