Human PDPN ELISA Kit

Cat#: orb563492 (ELISA Manual)

Catalogue No.: orb563492
Size: 48T/96T
Reactivity: Human
Range: 0.156-10 ng/ml
Sensitivity: < 0.094 ng/ml
Application: For quantitative detection of PDPN in serum, plasma, tissue homogenates and other biological fluids.
Storage: 4°C for 6 months

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications (48T/96T)	Storage
Micro ELISA Strip plate	8 x 6 or 8 x 12	4°C / -20°C
Lyophilised Standard	1 vial or 2 vials	4°C / -20°C
Sample / standard dilution buffer	10 ml / 20 ml	4°C
Biotin- detection antibody (Concentrated)	60 ul / 120 ul	4°C
Antibody dilution buffer	5 ml / 10 ml	4°C
HRP-Streptavidin Conjugate (SABC)	60 ul / 120 ul	4°C (Protect from light)
SABC dilution buffer	5 ml / 10 ml	4°C
TMB substrate	5 ml / 10 ml	4°C (Protect from light)
Stop solution	5 ml / 10 ml	4°C
Wash buffer (25X)	15 ml / 30 ml	4°C
Plate seal	3 / 5 seals	
Manual	1 copy	

Principle of the Assay

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Anti- PDPN antibody is pre-coated onto 96-well plates. The biotin conjugated anti- PDPN antibody is used as the detection antibody. The standards, test samples and biotin conjugated detection antibody are subsequently added to the wells, and washed with wash buffer. HRP Streptavidin is added and unbound conjugates are washed away with wash buffer. TMB substrates are used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colored product that changes to yellow after adding acidic stop solution. The intensity of the yellow color

is proportional to quantity of PDPN sample captured on the plate and therefore present in the sample. The O.D. absorbance at 450 nm is read on a microplate reader, and the concentration of PDPN can then be calculated.

Precautions for Use

1. To validate and optimize experimental protocol, sample dilution and applicability to application, it is recommended to carry out pilot experiments using standards and a small number of samples before carrying out experimental sample analysis.
2. After opening and before using, keep plate dry.
3. Before use centrifuge all tubes to bring down components to the bottom of the tubes.
4. Store TMB reagents out of direct light.
5. Washing processes are very important. Failure to adequately wash at appropriate steps can cause false positive results.
6. Duplicate well assay testing is recommended for both standards and samples.
7. Do not let the Micro plate dry completely at any point during the assay as this cause active components on the plate to become inactive.
8. Do not reuse tips or tubes in order to avoid cross contamination.
9. Avoid using reagents from different batches.

Material Required but Not Supplied

1. Microplate reader (wavelength: 450 nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Manual Plate Washing

Aspirate the solution from the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350 ul wash buffer and soak for 1 to 2 minutes. Aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure twice more for a total of THREE washes.

Automated Plate Washing

Aspirate all wells, then wash plate THREE times with 350 ul wash buffer each time. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Collection and Storage

Isolate the test samples soon after collecting then analyze immediately (within 2 hours). Alternatively, aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- **Serum:** Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- **Plasma:** Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Tissue homogenates:** For general information, hemolysed blood may affect the result. We recommend tissues are thoroughly rinsed with ice-cold PBS (0.01M, pH=7.4) to remove excess blood. Tissue pieces should be weighed and then minced before homogenization in PBS on ice, using a glass homogenizer. The volume of PBS used depends on the weight of the tissue. We recommend approximately 9 mL PBS to 1 gram tissue. It is recommended to add protease inhibitor to the PBS. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to generate the supernatant.
- **Cell culture supernatant:** Centrifuge supernatant for 20 minutes to remove insoluble impurities and cell debris at 1000×g at 2 - 8°C. Collect the clear supernatant and carry out the assay immediately.
- **Other biological fluids**: Centrifuge samples for 20 minutes at 1000×g at 2 - 8°C. Collect the supernatant and carry out the assay immediately.
- **Sample preparation:** Samples should be clear and transparent and be centrifuged to remove suspended solids.

Note:

Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Sample Dilution Guideline

End users should estimate the concentration of the target protein in the test sample first, and select an appropriate dilution factor to ensure the samples fall within the optimal detection range of the kit. Dilute the sample with the provided dilution buffer. The test sample must be well mixed with the dilution buffer. Standard curves and samples should be used in pilot experiments.

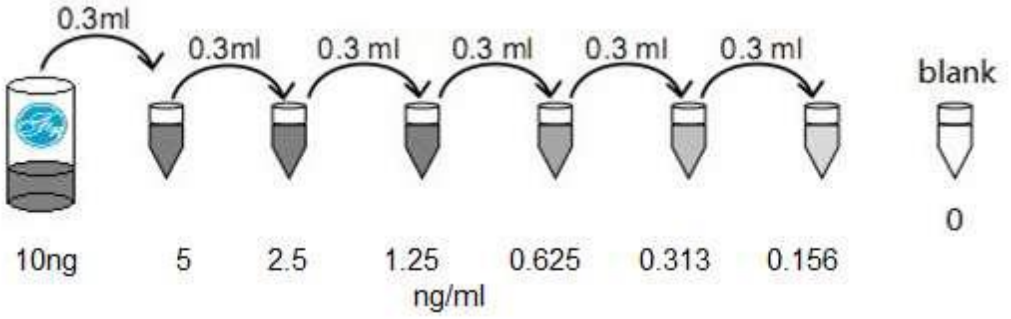
- High target protein concentration (100 - 1000 ng/ml): Dilution: 1:100. (i.e. Add 1 µl of sample into 99 µl of Sample / Standard dilution buffer.)
- Medium target protein concentration (10 - 100 ng/ml): Dilution: 1:10.(i.e. Add 10 µl of sample into 90 µl of Sample / Standard dilution buffer.)
- Low target protein concentration (0.156 - 10 ng/ml): Dilution: 1:2.(i.e. Add 50 µl of sample into 50 µl of Sample / Standard dilution buffer.)
- Very low target protein concentration (≤0.156 ng/ml): Unnecessary to dilute, or dilute at 1:2.

Reagent Preparation and Storage

Bring all reagents to room temperature before use.

1. Wash Buffer:
Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Store unused solution at 4°C. If crystals have formed in the concentrate, warm in a 40°C water bath (Heating temperature should not exceed 50°C) and mix gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2. Standard:
1) 10 ng/ml of standard solution: Add 1 ml of Sample / Standard dilution buffer to one Standard tube, keep the tube at room temperature for 10 min and mix thoroughly.
2) 5 ng/ml→0.156 ng/ml of standard solutions: Label 6 Eppendorf tubes as 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.3125 ng/ml, 0.156ng/ml, respectively. Aliquot 0.3 ml of the Sample / Standard dilution buffer into each tube. Add 0.3 ml of the above 10ng/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: The standard solutions are best used within 2 hours. The standard solution may be stored at 4°C for up to 12 hours. Alternatively, store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Preparation of Biotin- detection Antibody working solution

prepare within 1 hour of the experiment.
1) 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)
2) Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 µl of Biotin- detection antibody into 99 µl of Antibody dilution buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) working solution:

prepare within 30 mins of the experiment.
1) 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)

2) Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 µl of SABC into 99 µl of SABC dilution buffer.)

Assay Procedure

Before adding to wells, equilibrate the SABC working solution and TMB substrate for at least 30 min at room temperature (24°C). When diluting samples and reagents, they must be mixed completely and evenly. It is recommend to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommend to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (zero) wells!**
2. Aliquot 0.1 ml of 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.3125 ng/ml, 0.156 ng/ml, standard solutions into the standard wells.
3. Add 0.1 ml of Sample / Standard dilution buffer into the control (zero) well.
4. Add 0.1 ml of properly diluted sample (Human serum, plasma, tissue homogenates and other biological fluids.) into test sample wells.
5. Seal the plate with a cover and incubate at 37°C for 90 min.
6. Remove the cover and discard the plate content, clap the plate on the absorbent filter papers or other absorbent material. Do NOT let the wells completely dry at any time. Do Not Wash Plate!
7. Add 0.1 ml of Biotin- detection antibody working solution into the above wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37°C for 60 min.
9. Remove the cover, and wash plate 3 times with Wash buffer.
10. Add 0.1 ml of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
11. Remove the cover and wash plate 5 times with Wash buffer. Each time, incubate the wash buffer in the wells at RT for 1-2 min.
12. Add 90 µl of TMB substrate to each well, cover the plate and incubate at 37°C in dark for 15-30 min. (Note: This incubation time is for reference use only, the optimal time should be determined by end user). Blue colour should be visible in the first 3-4 wells of the plate (with most concentrated PDPN standard solutions but the other wells show no obvious color.
13. Add 50 µl of Stop solution to each well and mix thoroughly. The color should immediately change from blue to yellow.
14. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. For calculation, (the relative O.D 450) = (the O.D 450 of each well) – (the O.D 450 of Zero well). The standard curve can be plotted as the relative O.D 450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The PDPN concentration of the samples can be interpolated from the standard curve.

Note:

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

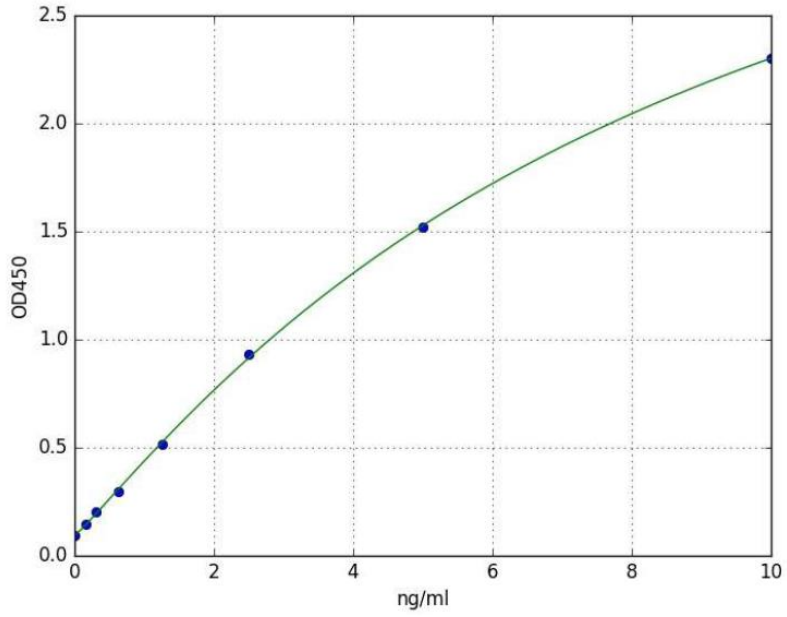
Summary

1. Wash plate 2 times before adding standard, sample and control (zero) wells!
2. Add 100 µL standard or sample to each well for 90 minutes at 37°C
3. add 100 µL Biotin- detection antibody working solution to each well for 60 minutes at 37°C
4. Aspirate and wash 3 times
5. Add 100 µL SABC working solution to each well. Incubate for 30 minutes at 37°C
6. Aspirate and wash 5 times
7. Add 90 µL TMB substrate. Incubate 15 -30 minutes at 37°C
8. Add 50 µL Stop Solution. Read at 450nm immediately
9. Calculation of results

Typical Data & Standard Curve

Results of a typical standard run of a PDPN ELISA Kit are shown below. This standard curve was generated for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	ng/ml	0	0.156	0.312	0.625	1.25	2.5	5	10
Y	OD450	0.092	0.145	0.203	0.296	0.516	0.931	1.519	2.304



Specificity

This assay has high sensitivity and excellent specificity for detection of PDPN . No significant cross-reactivity or interference between PDPN and analogues was observed.
Note: Limited by current skills and knowledge, it is impossible for us to complete the cross - reactivity detection between PDPN and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with known level of PDPN and the recovery rates were calculated by comparing the measured value to the expected amount of PDPN in samples.

Matrix	Recovery range (%)	Average(%)
serum(n=5)	85-100	93
EDTA plasma(n=5)	90-102	94
heparin plasma(n=5)	85-97	93

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of PDPN and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	88-103%	91-104%	90-102%	85-103%
EDTA plasma(n=5)	85-90%	83-95%	85-100%	93-101%
heparin plasma(n=5)	81-92%	82-100%	82-98%	85-97%

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level PDPN were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level PDPN were tested on 3 different plates, 8 replicates in each plate.
CV (%) = SD/meanX100
Intra-Assay: CV<8%
Inter-Assay: CV<10%

Stability

The stability of the ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

Standard(n=5)	37°C for 1 months	4°C for 6 months
Average(%)	80	95-100

To minimize external influence on the performance of the kit; operation procedures and lab conditions, particularly room temperature, air humidity and incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.