Human PROZ(Protein Z) ELISA Kit

Catalog No: AAA21705

96T/48T/24T

This manual must be read attentively and completely before using this product.
Please refer to specific expiry date from label on the side of box.
If you have any problems, please contact our Technical Service Center for help.

Intended use

This ELISA kit applies to the in vitro quantitative determination of Human PROZ concentrations in plasma sample.

Specification

- Sensitivity: 0.1ng/mL.
- Detection Range: 0.16-10ng/mL
- Specificity: This kit recognizes natural and some recombinant Human PROZ. No Significant cross-reactivity or interference between Human PROZ and analogues was observed.
- Repeatability: Coefficient of variation is < 10%.

Test principle

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human PROZ. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human PROZ and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human PROZ, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human PROZ. You can calculate the concentration of Human PROZ in the samples by comparing the OD of the samples to the standard curve.

Kit components & Storage

An unopened kit can be stored at 4℃ for 1 month. If the kit is not used within 1 month, store the items separately according to the following conditions once the kit is received.

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips	-20℃, 6 months
Reference Standard	96T: 2 vials 48T: 1 vial 24T: 1 vial	
Concentrated Biotinylated Detection Ab (100×)	96T: 1 vial, 120 µL 48T: 1 vial, 60 µL 24T: 1 vial, 60 µL	
Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 µL 48T: 1 vial, 60 µL 24T: 1 vial, 60 µL	-20℃(Protect from light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	4℃, 6 months
Biotinylated Detection Ab Diluent	1 vial, 14 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	4℃(Protect from light)
Stop Solution	1 vial, 10 mL	4℃
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other supplies required

Microplate reader with 450 nm wavelength filter

High-precision transfer pipette, EP tubes and disposable pipette tips

Incubator capable of maintaining 37℃

Deionized or distilled water

Absorbent paper

Loading slot for Wash Buffer

Note

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA Plate may appear to have a water-like substance, which is normal and will not have any impact on the experimental results.
3. Do not reuse the reconstituted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wave length at 450±10 nm. The optical density should be within 0-3.5. Follow the Instructions of the Microplate Reader for set-up and preheat it for 15 min before OD measurement.
5. Do not mix or use components with those from other lots.
6. Change pipette tips in between adding standards, in between sample additions, and in between reagent additions. Also, use separate reservoirs for each reagent.

Sample collection

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8℃ within 30 min of collection. Collect the supernatant to carry out the assay. Samples with high hemolysis or much lipid are not suitable for ELISA assay!

Note for sample

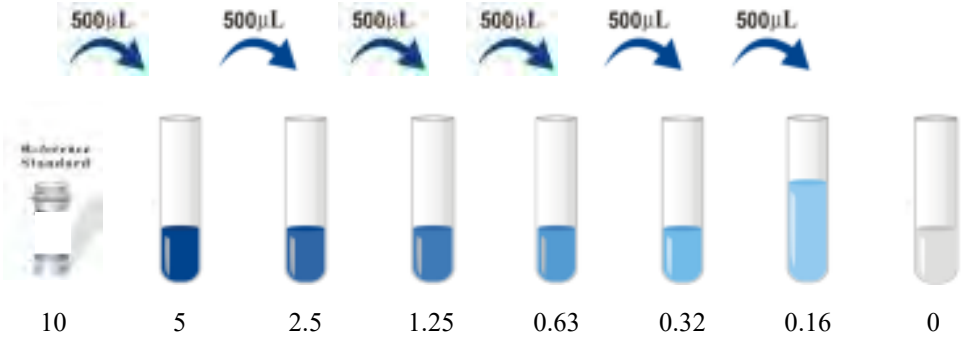
1. Samples should be assayed within 7 days when stored at 4℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles.
2. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
4. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.
5. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer**: Dilute 30 mL of Concentrated Wash Buffer with deionized or distilled water to prepare 750 mL Wash Buffer. Note: if crystals have formed in the concentrate, warm it in 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 10ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 10、5、2.5、1.25、0.63、0.32、0.16、0 ng/mL.

   Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 10ng/mL working solution to the first tube and mix up to produce a 5ng/mL working solution. Pipette 500uL of the

solution from former tube to the latter one in order according to this step. The illustration below is for reference. Note: the last tube is regarded as blank. Don't pipette solution to it from the former tube.



4. **Biotinylated Detection Ab working solution:** Calculate the required amount before experiment (100 μL/well). In actual preparation, more account of 100~200μLshould be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.
5. **Concentrated HRP Conjugate working solution:** Calculate the required amount before experiment (100 μL/well). In actual preparation, more account of 100~200 μL should be prepared. Dilute the 100× Concentrated HRP Conjugate to 1× working solution with Concentrated HRP Conjugate Diluent.

Assay procedure (A brief assay procedure is on the 7th page**)**

1. Add **Standard working solution** of different concentrations to the first two columns: Each concentration of the solution is added into two wells side by side (100 uL for each well). Add samples to other wells (100 uL for each well). Cover the plate with sealer provided in the kit. Incubate for 90 min at 37°C. Note: solutions should be added to the bottom of micro ELISA plate well, avoid touching the inside wall and foaming as possible.
2. Remove the liquid of each well, do not wash. Immediately add 100 μL of **Biotinylated Detection Ab working solution** to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37°C.
3. Aspirate or decant the solution from each well， add 350 uL of **wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
4. Add 100 μL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3.
6. Add 90 μL of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.
7. Add 50 μL of **Stop Solution** to each well. Note: the order to add stop solution should be the same as the substrate solution.
8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450nm.

Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

Typical data

As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish standard curve for each test. Typical standard curve and data below is provided for reference only.

Concentration(ng/mL)	10	5	2.5	1.25	0.63	0.32	0.16	0
OD	2.534	1.679	1.025	0.531	0.301	0.189	0.134	0.078
Corrected OD	2.456	1.601	0.947	0.453	0.223	0.111	0.056	-

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Human PROZ were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Human PROZ were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
mean(ng/mL)	0.46	0.85	3.76	0.47	0.78	3.6
Standard deviation	0.03	0.04	0.14	0.03	0.05	0.17
CV (%)	6.52	4.71	3.72	6.38	6.41	4.72

Recovery

The recovery of Human PROZ spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Range (%)	Average Recovery (%)
EDTA plasma (n=5)	93-107	99

Linearity

Samples were spiked with high concentrations of Human PROZ and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		EDTA plasma (n=5)
1:2	Range (%)	88-102
	Average (%)	95
1:4	Range (%)	81-93
	Average (%)	86
1:8	Range (%)	80-94
	Average (%)	87
1:16	Range (%)	87-100
	Average (%)	93

Troubleshooting

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells between steps.
Low signal	Insufficient incubation time	Ensure sufficient incubation time.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation.
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting in the Microplate reader.
		Open the Microplate Reader ahead to pre-heat.
Large CV	Inaccurate pipetting	Check pipettes.
High background	Concentration of target protein is too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

for research use only

SUMMARY

1. Add 100 µL standard or sample to each well. Incubate for 90 min at 37℃.
2. Remove the liquid. Add 100 µL Biotinylated Detection Ab. Incubate for 1 hour at 37℃.
3. Aspirate and wash 3 times.
4. Add 100 µL HRP Conjugate. Incubate for 30 min at 37℃.
5. Aspirate and wash 5 times.
6. Add 90 µL Substrate Reagent. Incubate for 15 min at 37℃.
7. Add 50 µL Stop Solution. Read at 450 nm immediately.
8. Calculation of results.

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on. We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipments, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven’t compared our products with those from other manufacturers.
9. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.