ab108890 – Prothrombin Pig ELISA Kit

Instructions for Use

For the quantitative measurement of Pig Prothrombin in plasma, serum, cell culture, cell lysate, and tissue samples.

View kit datasheet: www.abcam.com/ab108890

(use www.abcam.cn/ab108890for China, or www.abcam.co.jp/ab108890 for Japan)

This product is for research use only and is not intended for diagnostic use.

Version 2b Last Updated 26 January 2024

Table of Contents

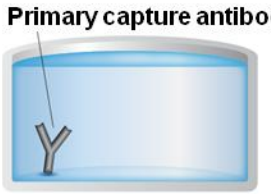
1. BACKGROUND

Abcam's Prothrombin Pig *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of prothrombin levels in plasma, serum, cell culture, cell lysate, and tissue samples.

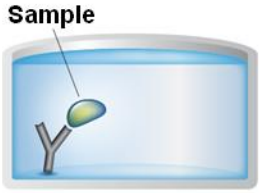
A Prothrombin specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently a Prothrombin specific biotinylated detection antibody is added and then followed by washing with wash buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of Prothrombin captured in plate.

Prothrombin is also known as Factor II. The conversion of Factor X to Xa changes prothrombin into its active form, thrombin, which then accelerates the formation of fibrin. The level of the plasma prothrombin in the circulating blood decreases during its passage through the pulmonary capillaries. The bleeding tendency in acute chloroform intoxication is due to deficiency in both plasma fibrinogen and plasma prothrombin. On the other hand, in severe Alzheimer's disease, prothrombin was localized within the wall and neuropil surrounding microvessels. It has been reported that plasma prothrombin level increases in sepsis patients, and in chronic gastrointestinal disorders.

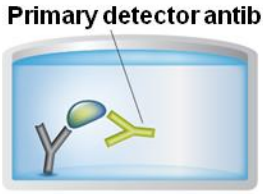
2. ASSAY SUMMARY



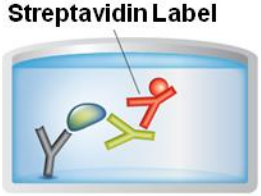
Prepare all reagents, samples and standards as instructed.



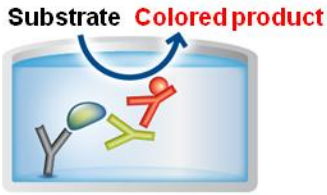
Add standard or sample to each well used. Incubate at room temperature.



Wash and add prepared biotin antibody to each well. Incubate at room temperature.



Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.



Add Chromogen Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 4°C immediately upon receipt, apart from the SP Conjugate & Biotinylated Antibody, which should be stored at -20°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Prothrombin Microplate (12 x 8 well strips)	96 wells	4°C
Prothrombin Standard	1 vial	-20°C
10X Diluent N Concentrate	30 mL	4°C
Biotinylated Pig Prothrombin Antibody	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	7 mL	4°C
Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Sealing Tapes	3	N/A

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 7 tubes to prepare standard or sample dilutions.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare fresh reagents immediately prior to use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

9.1 **1X Diluent N**

Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. *Store for up to 1 month at 4°C.*

9.2 **1X Wash Buffer**

Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

9.3 **1X Biotinylated Prothrombin Detector Antibody**

9.3.1 The stock Biotinylated Prothrombin Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated Prothrombin Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated Prothrombin Antibody.

9.3.2 Calculate the necessary amount of 1X Diluent N to dilute the Biotinylated Prothrombin Antibody to prepare a 1X Biotinylated Prothrombin Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

Number of Wells Strips	Number of Wells	(V_T) Total Volume of 1X Biotinylated Antibody (µL)
4	32	1,760
6	48	2,640
8	64	3,520
10	80	4,400
12	96	5,280

Any remaining solution should be frozen at -20°C.

Where:

C_S = Starting concentration (X) of stock Biotinylated Prothrombin Antibody (variable)

C_F = Final concentration (always = 1X) of 1X Biotinylated Prothrombin Antibody solution for the assay procedure

V_T = Total required volume of 1X Biotinylated Prothrombin Antibody solution for the assay procedure

V_A = Total volume of (X) stock Biotinylated Prothrombin Antibody

V_D = Total volume of 1X Diluent N required to dilute (X) stock Biotinylated Prothrombin Antibody to prepare 1X Biotinylated Prothrombin solution for assay procedures

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells:

$$(C_F / C_S) \times V_T = V_A$$

Calculate the final volume of 1X Diluent N required to prepare the 1X Biotinylated Prothrombin Antibody:

$$V_T - V_A = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your antibody vial for the actual concentration of antibody provided.

C_S = 50X Biotinylated Prothrombin Antibody stock

C_F = 1X Biotinylated Prothrombin Antibody solution for use in the assay procedure

V_T = 3,520 µL (8 well strips or 64 wells)

$$(1X/50X) \times 3{,}520\ \mu L = 70.4\ \mu L$$

$$3{,}520\ \mu L - 70.4\ \mu L = 3{,}449.6\ \mu L$$

V_A = 70.4 µL total volume of (X) stock Biotinylated Prothrombin Antibody required

V_D = 3,449.6 µL total volume of 1X Diluent N required to dilute the 50X stock Biotinylated Antibody to prepare 1X Biotinylated Prothrombin Antibody solution for assay procedures

9.3.3 First spin the Biotinylated Prothrombin Antibody vial to collect the contents at the bottom.

9.3.4 Add calculated amount V_A of stock Biotinylated Prothrombin Antibody to the calculated amount V_D of 1X Assay Diluent N. Mix gently and thoroughly.

9.4 **1X SP Conjugate**

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N.

Any remaining solution should be frozen at -20°C.

10. STANDARD PREPARATIONS

- **Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.**
- **Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.**
- **This procedure prepares sufficient standard dilutions for duplicate wells.**

10.1 Reconstitution of the Prothrombin Standard vial to prepare a 400 ng/mL Prothrombin **Stock Standard**:

10.1.1 First consult the Prothrombin Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent M to add when resuspending the Prothrombin Standard vial to produce a 400 ng/mL Prothrombin **Stock Standard** by using the following equation:

C_S = Starting mass of Prothrombin Standard (see vial label) (ng)
C_F = 400 ng/mL Prothrombin **Stock Standard** final required concentration
V_D = Required volume of 1X Diluent M for reconstitution (µL)

Calculate total required volume 1X Diluent M for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 440 ng of Prothrombin Standard in vial

C_F = 400 ng/mL Prothrombin **Stock Standard** final concentration

V_D = Required volume of 1X Diluent M for reconstitution

$$(440 \text{ ng} / 400 \text{ ng/mL}) \times 1{,}000 = 1{,}100 \text{ µL}$$

10.1.3 First briefly spin the Prothrombin Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the Prothrombin Standard vial by adding the appropriate calculated amount V_D of 1X Diluent M to the vial to generate the 400 ng/mL Prothrombin **Stock Standard**. Mix gently and thoroughly.

10.2 Allow the reconstituted 400 ng/mL Prothrombin **Stock Standard** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

10.3 Label eight tubes #1-8.

10.4 Prepare the 100 ng/mL **Standard #1** by adding 120 µL of the reconstituted 400 ng/mL Prothrombin **Stock Standard** to 360 µL of 1X Diluent M and mix thoroughly and gently.

10.5 Add 360 µL of 1X Diluent M to tubes #2 – 8.

10.6 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

10.7 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

10.8 Using the table below as a guide, prepare subsequent serial dilutions.

10.9 1X Diluent M serves as the zero standard, 0 ng/mL (tube #8).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Volume Diluent N (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Step 10.1				400
2	120	360	480	400	100
3	120	360	480	100	25
4	120	360	480	25	6.250
5	120	360	480	6.25	1.563
6	120	360	480	1.563	0.391
7	-	360	360	-	0

11. SAMPLE PREPARATION

11.1 Plasma

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x *g* for 10 minutes. Plasma dilution is suggested at 1:12,000 in 1X Diluent N then assay; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (Heparin and EDTA can also be used as an anticoagulant).

11.2 Cell Culture Supernatants

Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into 1X Diluent N; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

11.3 Serum

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x g for 10 minutes and remove serum. Serum dilution is suggested at 1:12,000 in 1X Diluent N then assay; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.4 Cell Lysate

Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (PBS, 1% Triton X-100, protease inhibitor cocktail). For every 1 x 106 cells, add approximately 100 µl of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. If necessary,

dilute samples into 1X Diluent N; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

11.5 **Tissue**

Extract tissue samples with 0.1 M phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. If necessary, dilute samples into 1X Diluent N; user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).

13.2 Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.

13.3 Add 50 µL of Prothrombin standard or sample to each well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.

13.4 Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µl of Wash Buffer per well. Invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a microplate washer, wash six times with 300 µl of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.

13.5 Add 50 µL of 1X Biotinylated Prothrombin Antibody to each well and incubate for one hour.

13.6 Wash microplate as described above.

13.7 Add 50 µL of 1X SP Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

13.8 Wash microplate as described above.

13.9 Add 50 µL of Chromogen Substrate per well and incubate in ambient light for 10 minutes or until the optimal blue colour density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

13.10 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.

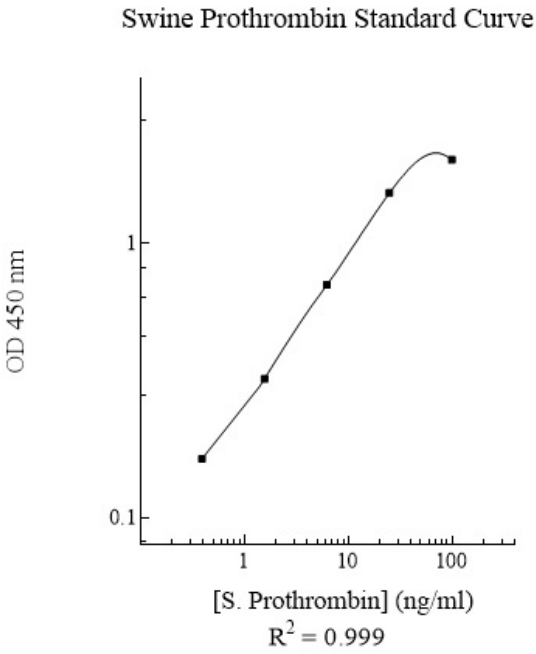
13.11 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

14. CALCULATIONS

Calculate the mean value of the triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of Prothrombin is typically ~0.23 ng/mL.

RECOVERY –

Standard Added Value: 1.563 – 25 ng/mL

Recovery %: 90 – 112.

Average Recovery %: 98

LINEARITY OF DILUTION –

Average % Expected Value		
Sample Dilution	**Plasma**	**Serum**
1:6000	110%	108%
1:12000	94%	100%
1:24000	90%	91%

PRECISION –

	Intra-Assay	**Inter-Assay**
% CV	3.1	10.5

17. ASSAY SPECIFICITY

Species	% Cross Reactivity
Canine	0
Bovine	0
Equine	0
Monkey	0
Mouse	0
Rat	0
Rabbit	0
Swine	100
Human	0

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4ºC
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background/ Low sensitivity	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

19. NOTES

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)