Swine Prothrombin ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

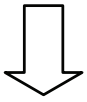
The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

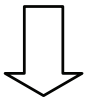
Thank you for choosing Assaypro.

Assay Summary

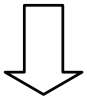
Add 50 µl of standard/sample per well.
Incubate 2 hours.



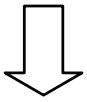
Wash, then add 50 µl of
biotinylated antibody per well.
Incubate 1 hour.



Wash, then add 50 µl of SP per well.
Incubate 30 minutes.



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 15 minutes.



Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
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AssayMax Swine Prothrombin ELISA Kit

Catalog No. EPP3022-1
Sample Insert/Reference Only

Introduction

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 (1). The bleeding tendency in acute chloroform intoxication is due to deficiency in both plasma fibrinogen and plasma prothrombin (2). On the other hand, in severe Alzheimer's disease, prothrombin was localized within the wall and neuropil surrounding microvessels (3). It has been reported that plasma prothrombin level increases in sepsis patients (4), and in chronic gastrointestinal disorders (5).

Principle of the Assay

The AssayMax Swine Prothrombin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of swine prothrombin in plasma, serum, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures swine prothrombin in less than 4 hours. A monoclonal antibody specific for swine prothrombin has been pre-coated onto a 96-well microplate with removable strips. Prothrombin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for swine prothrombin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The stop solution is an acidic solution.

Reagents

- **Swine Prothrombin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against swine prothrombin.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Swine Prothrombin Standard:** Swine prothrombin in a buffered protein base (800 ng, lyophilized).
- **Biotinylated Swine Prothrombin Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against swine prothrombin (80 µl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- Store SP conjugate and biotinylated antibody at -20°C.
- Store microplate, diluent concentrate (10x), wash buffer, stop solution, and chromogen substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.

- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **Plasma:** Collect swine plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Plasma dilution is suggested at 1:12000 in EIA diluent; however, the user should determine the optimal dilution factor, and assay. 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).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes. Serum dilution is suggested at 1:12000 in EIA diluent; however, the user should determine the optimal dilution factor, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 800 ng of Swine Prothrombin Standard with 2 ml of EIA diluent to generate a stock solution of 400 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (400 ng/ml) 1:4 with EIA diluent to produce 100, 25, 6.25, 1.563, and 0.391 ng/ml solutions. EIA diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Swine Prothrombin] (ng/ml)
P1	Standard (400 ng/ml)	400.0
P2	1 part P1 + 3 parts EIA Diluent	100.0
P3	1 part P2 + 3 parts EIA Diluent	25.00
P4	1 part P3 + 3 parts EIA Diluent	6.250
P5	1 part P4 + 3 parts EIA Diluent	1.563
P6	1 part P5 + 3 parts EIA Diluent	0.391
P7	EIA Diluent	0.000

- **Biotinylated Swine Prothrombin Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Swine Prothrombin Standard or sample per well, cover wells, and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of wash buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of wash buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Swine Prothrombin Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.

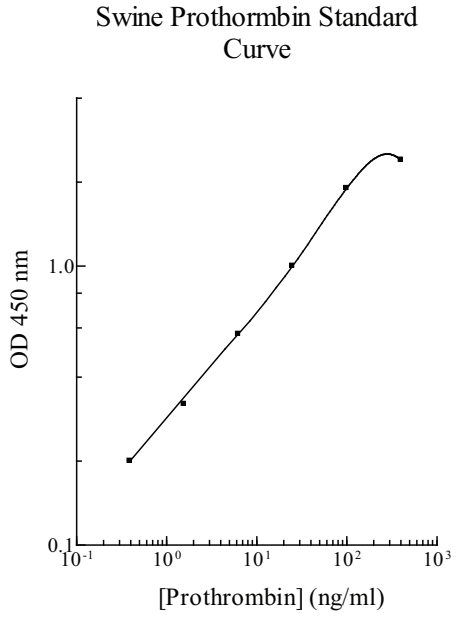
- Add 50 µl of Chromogen Substrate per well and incubate for about 15 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- 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.

Data Analysis

- Calculate the mean value of the duplicate or 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 4-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of Swine Prothrombin is ~ 0.35 ng/ml
- Intra-assay and inter-assay coefficients of variation were 4.8% and 7.4% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:12000	95%	94%
1:24000	91%	89%
1:48000	86%	88%

Recovery

Standard Added Value	1- 100 ng/ml
Recovery %	82 - 104%
Average Recovery %	97%

Cross-Reactivity

Species	% Cross Reactivity
Canine	0.05%
Bovine	0.005%
Monkey	0.05%
Mouse	0.1%
Rat	0.005%
Rabbit	0.1%
Swine	100%
Human	0.05%

References

(1) William DE W. Andrus *et al.* (1940) *Science* 91, 2350, 48 – 50
(2) H. P. Smith *et al.* (1937) *The Journal of Experimental Medicine* 66, 801-811,
(3) Zipser BD *et al.* (2006) *Neurobiol Aging*. June 15
(4) Hesselvik JF. (1987) *Crit Care Med.* Dec; 15(12): 1092-7
(5) Krasinski SD *et al.* (1985) *Am J Clin Nutr.* Mar; 41(3): 639-43

Version 1.3R3

Related Products

- ET4010-1 AssayMax Human Thrombin ELISA Kit (Cell Culture samples)
- EP3022-1 AssayMax Human Prothrombin ELISA Kit (Plasma, Milk, Urine, and Cell Culture samples)
- EMP3022-1 AssayMax Mouse Prothrombin ELISA Kit (Plasma, Serum, Urine, and Cell Culture samples)