F2 (Pig) ELISA Kit

Catalog Number KA1407

96 assays

Version: 04

Intended for research use only

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Introduction

Background

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 prothormbin level increases in sepsis patients (4), and in chronic gastrointestinal disorders (5).

Principle of the Assay

The F2 (Pig) ELISA Kit is designed for detection of Prothrombin in swine plasma, serum and cell culture, and cell lysate samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures swine Prothrombin in approximately 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 (SP) 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.

General Information

Materials Supplied

List of component

Component	Amount
Swine Prothrombin Microplate: A 96-well polystyrene microplate coated with a monoclonal antibody against swine prothrombin.	96 (8x12) wells
Sealing Tapes: Pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Swine Prothrombin Standard: Swine prothrombin in a buffered protein base, lyophilized.	520 ng
Biotinylated Swine Prothrombin Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against swine prothrombin.	120 µL
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 mL
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 mL x 2
SP Conjugate (100x): A 100-fold concentrate.	80 µL
Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 mL
Stop Solution (1x): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction.	12 mL

Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures 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.
- ✓ Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel pipettes).
- ✓ Deionized or distilled reagent grade water.

Precautions for Use

- ✓ This product is for Research Use Only and is not intended for use in diagnostic procedures.
- ✓ Prepare all reagents (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.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acidic solution.

Assay Protocol

Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use.
- ✓ MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.
- ✓ Swine Prothrombin Standard: Reconstitute the Swine Prothrombin Standard (520 ng) with 1.3 mL of MIX Diluent to generate a 400 ng/mL of standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (400 ng/mL) 4-fold with MIX Diluent to produce 100, 25, 6.25, 1.563, and 0.391 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining stock solution should be frozen at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[Prothrombin] (ng/mL)
P1	1 part Standard (400 ng/mL)+ 3 parts MIX Diluent	100.00
P2	1 part P1 + 3 parts MIX Diluent	25.00
P3	1 part P2 + 3 parts MIX Diluent	6.250
P4	1 part P3 + 3 parts MIX Diluent	1.563
P5	1 part P4 + 3 parts MIX Diluent	0.391
P6	MIX Diluent	0.000

- ✓ Biotinylated Swine Prothrombin Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent to produce a 1x solution. The undiluted antibody should be stored 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 20-fold with reagent grade water to produce a 1x solution.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Sample Preparation

- Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. A 12000-fold sample dilution is suggested into MIX Diluent; however, the user should determine the optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 12000-fold sample dilution is suggested into MIX Diluent; however, the user should determine the optimal dilution factor depending on application needs. 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 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.
- 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 (10 mM Tris pH 8.0, 130 mM NaCl, 1% Triton X-100, protease inhibitor cocktail). For every 1 x 10^6 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. Samples can be stored 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.

✓ Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater (for reference only; please follow the insert for specific dilution suggested)	
100x	10000x
A) 4 µL sample: 396 µL buffer (100x) = 100-fold dilution Assuming the needed volume is less than or equal to 400 µL.	A) 4 µL sample: 396 µL buffer (100x) B) 4 µL of A: 396 µL buffer (100x) = 10000-fold dilution Assuming the needed volume is less than or equal to 400 µL.
1000x	100000x
A) 4 µL sample: 396 µL buffer (100x) B) 24 µL of A: 216 µL buffer (10x) = 1000-fold dilution Assuming the needed volume is less than or equal to 240 µL.	A) 4 µL sample: 396 µL buffer (100x) B) 4 µL of A: 396 µL buffer (100x) C) 24 µL of B: 216 µL buffer (10x) = 100000-fold dilution Assuming the needed volume is less than or equal to 240 µL.

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
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.

3. Add 50 µL of Swine prothrombin standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
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 each time and decant the contents; hit 4-5 times on absorbent material 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.
5. Add 50 µL of Biotinylated Swine Prothrombin Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 µL of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 12 minutes or until the optimal blue color density develops.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
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.

✓ Assay Summary

1. Add 50 µL of Standard or Sample per well. Incubate 2 hours.
2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour.
3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.
4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 12 minutes.
5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.

Data Analysis

Calculation of Results

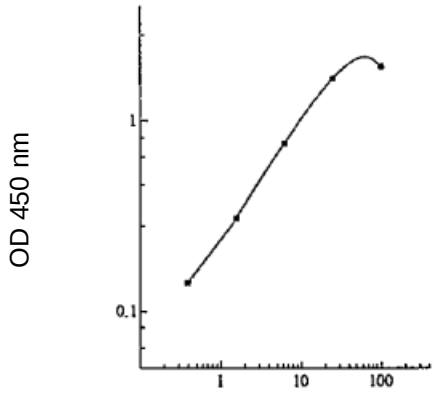
- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression 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.
- ✓ Typical Data

  The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/mL	OD	Average OD
P1	100	1.904 1.932	1.918
P2	25	1.681 1.639	1.660
P3	6.25	0.768 0.754	0.761
P4	1.563	0.310 0.304	0.307
P5	0.391	0.143 0.139	0.141
P6	0.000	0.083 0.085	0.084
Sample: Pooled Sodium Citrate Plasma (12000x)		1.002 1.026	1.014

- ✓ 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 as calculated by 2SD from the mean of a zero standard was established to be 0.24 ng/mL.
- ✓ Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
- ✓ Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	4.4%	4.7%	5.2%	9.3%	9.7%	9.9%
Average CV (%)	4.8%			9.6%		

- Recovery

Standard Added Value	1.563 – 25 ng/mL
Recovery %	90-112 %
Average Recovery %	98 %

- Linearity

  Plasma and serum samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
6000x	94%	95%
12000x	99%	98%
24000x	96%	93%

- Cross-Reactivity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Monkey	None
Mouse	None
Rat	None
Rabbit	None
Human	None

Resources

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are empty after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.

Deficient Standard Curve Fit	Non-optimal sample dilution	• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. • Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. • User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the aluminum sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

References

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Plate Layout

12								
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10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H