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Porcine Fibrinogen ELISA Kit

Strip well format. Reagents for up to 96 tests

Catalog No.	**CS365A** **CS365B**	**Quantity:**	**1 x 96 tests** **5 x 96 tests**

Intended Use: This porcine fibrinogen antigen assay is intended for the quantitative determination of total fibrinogen antigen in porcine plasma and serum/

Background: Fibrinogen is a soluble glycoprotein that circulates in the blood and is converted to insoluble fibrin by thrombin in the final step of the coagulation cascade. Hepatic expression of fibrinogen increases two to four hundred fold during the acute phase response to infection or inflammation. Elevated fibrinogen levels are correlated with cardiovascular disease and atherosclerosis.

Assay Principle: Porcine fibrinogen will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled polyclonal anti-porcine fibrinogen primary antibody binds to the captured protein. Excess antibody is washed away and bound polyclonal antibody is reacted with streptavidin conjugated to horseradish peroxidase. TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of porcine fibrinogen. Color development is proportional to the concentration of fibrinogen in the samples.

Reagents Provided:

♦96-well microtiter strip plate
8 X12 well removable strips containing affinity purified anti-porcine fibrinogen antibody dried and blocked on the surface.

♦5X Diluent
1 bottle of 50 mL; bring to 1X using DI water

♦10X Wash Buffer
1 bottle of 50 mL; bring to 1X using DI water

♦Porcine fibrinogen standard
1 vial of lyophilized standard

♦Anti-porcine fibrinogen primary antibody
1 vial of lyophilized biotinylated antibody

♦Peroxidase conjugated avidin
1 vial of concentrated HRP-labeled avidin

♦TMB substrate solution
1 bottle of 10 mL solution

♦Stop solution
1 bottle of 6 mL 1 M sulfuric acid

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Storage and Stability:

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

Reagents and Equipment Required:

- 1-channel pipettes covering 0-10 µl and 200-1000 µl
- 12-channel pipette covering 30-300 µl
- Paper towels or kimwipes
- 50 ml tubes, 1.5 ml centrifuge tubes
- 1 N H_2SO_4
- Magnetic stirrer and stir-bars
- DI water
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm. (Optional)

Warnings:

Warning – Avoid skin and eye contact when using TMB substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

Precautions:

- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Preparation of Reagents:

- **Diluent concentrate:** The diluent is supplied in a 5X concentrate and must be diluted 1:5 with deionized water for use with the kit.
- **Wash buffer concentrate:** The wash buffer is supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

Specimen Collection:

The assay measures total porcine fibrinogen in the 1.56-800 ng/ml range. Samples giving porcine fibrinogen levels above 500 ng/ml should be diluted in 1X diluent before use. A 1:500,000 to 1:1,000,000 dilution for normal plasma is suggested for best results.

Assay Procedure:

Perform assay at room temperature. Vigorously shake plate (300 rpm) at each step of the assay. If a microtiter plate shaker is not available then keep the plate on a flat surface for 60 minutes at each step instead of 30 minutes.

Note: When the assay is performed without shaking the plate, the final absorbance values at 450 nm will be lower than when the assay is performed using a plate shaker.

Preparation of Standard:

Reconstitute standard with 5 ml of 1X diluent and give a 800 ng/ml solution.

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Dilution table:

Fibrinogen concentration (ng/ml)	Dilutions
800	Straight from the vial
400	500µl (1X diluent) + 500µl (800ng/ml)
200	500µl (1X diluent) + 500µl (400ng/ml)
100	500µl (1X diluent) + 500µl (200ng/ml)
50	500µl (1X diluent) + 500µl (100ng/ml)
25	500µl (1X diluent) + 500µl (50ng/ml)
12.5	500µl (1X diluent) + 500µl (25ng/ml)
6.25	500µl (1X diluent) + 500µl (12.5ng/ml)
3.125	500µl (1X diluent) + 500µl (6.25ng/ml)
1.56	500µl (1X diluent) + 500µl (3.125ng/ml)
0	500µl (1X diluent) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100 µl standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Primary Antibody Addition:
Add 10 ml of 1X diluent directly to the primary antibody vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

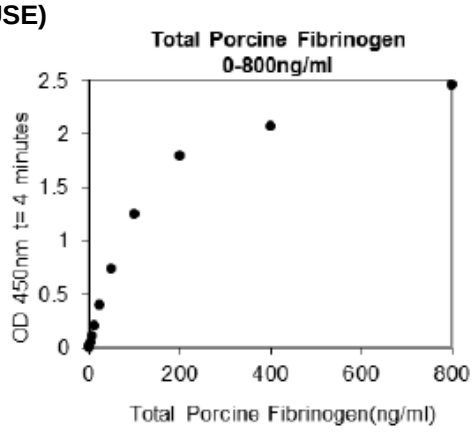
Avidin-HRP Addition:
Dilute 2µl of HRP conjugated avidin into 10 ml of 1X diluent for a 1:5,000 dilution and add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

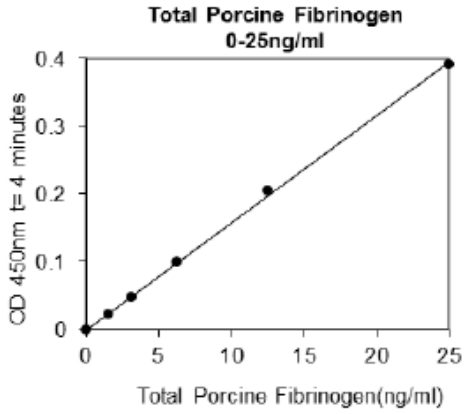
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance values at 450 nm. For best results read plate immediately.

Measurement:
Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450 nm, A_{450}. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:
Plot A_{450} against the amount of porcine fibrinogen in the standards. Fit a straight line through the linear points of the standard curve points using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of total porcine fibrinogen in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





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Expected Values: Fibrinogen is present in normal porcine plasma at a concentration of 2-4 mg/ml. The minimum detectable dose (MDD) of porcine fibrinogen is 0.6 ng/ml. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero point replicates and calculating the corresponding concentration.

Disclaimer: This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

Example of ELISA Kit Plate Layout
96 Well Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	1.56ng/ml	3.125ng/ml	6.25ng/ml	12.5ng/ml	25ng/ml	50ng/ml	100ng/ml	200ng/ml	400ng/ml	800ng/ml	
B	0	1.56ng/ml	3.125ng/ml	6.25ng/ml	12.5ng/ml	25ng/ml	50ng/ml	100ng/ml	200ng/ml	400ng/ml	800ng/ml	
C												
D												
E												
F												
G												
H												

Standards: 22 wells
Samples: 74 wells

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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