

# **LSBio™ Porcine Fibrinogen ELISA Kit**

**Catalog No. LS-F10455**

## **User Manual**

**Please Read the Manual Carefully  
Before Starting your Experiment**



For research use only. Not approved for use in humans or for clinical diagnosis.

### INTENDED USE

This porcine fibrinogen antigen assay is intended for the quantitative determination of total fibrinogen antigen in porcine plasma and serum. For research use only.

### 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 [1]. Hepatic expression of fibrinogen increases two to four hundred fold during the acute phase response to infection or inflammation [2]. Elevated fibrinogen levels are correlated with cardiovascular disease [3] and atherosclerosis [4].

### 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 antibody coated microtiter strip plate** (removable wells 8x12) containing anti-porcine fibrinogen antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **5X Diluent:** 1 bottle of 50ml
- **Porcine fibrinogen standard:** 1 vial lyophilized standard
- **Anti-porcine fibrinogen primary antibody antibody:** 1 vial lyophilized polyclonal antibody
- **TMB substrate solution:** 1 bottle of 10ml
- **Stop Solution:** 1 bottle 6ml of sulfuric acid

### STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard may be stored at -80°C for later use. Do not freeze-thaw the standard more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

### OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H<sub>2</sub>SO<sub>4</sub> or 1N HCl

### PRECAUTIONS

- **FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.**
- 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.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

### PREPARATION OF REAGENTS

- **1X Diluent:** 5X Diluent may contain precipitate. Warm to redissolve before use. Dilute 50ml of 5X diluent concentrate with 200ml of deionized water.
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.



## SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

## ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

### Preparation of Standard

Reconstitute standard by adding 5ml of diluent directly to the vial and agitate gently to completely dissolve contents. This will result in a 800ng/ml standard solution.

Dilution table for preparation of porcine fibrinogen standard:

Fibrinogen concentration (ng/ml)	Dilutions
800	Straight from the vial
400	500 $\mu\text{l}$ Diluent + 500 $\mu\text{l}$ (800ng/ml)
200	600 $\mu\text{l}$ Diluent + 400 $\mu\text{l}$ (400ng/ml)
100	500 $\mu\text{l}$ Diluent + 500 $\mu\text{l}$ (200ng/ml)
50	500 $\mu\text{l}$ Diluent + 500 $\mu\text{l}$ (100ng/ml)
25	600 $\mu\text{l}$ Diluent + 400 $\mu\text{l}$ (50ng/ml)
12.5	500 $\mu\text{l}$ Diluent + 500 $\mu\text{l}$ (25ng/ml)
6.5	500 $\mu\text{l}$ Diluent + 500 $\mu\text{l}$ (12.5ng/ml)
3.125	600 $\mu\text{l}$ Diluent + 400 $\mu\text{l}$ (6.25ng/ml)
1.56	500 $\mu\text{l}$ Diluent + 500 $\mu\text{l}$
0	500 $\mu\text{l}$ 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 and add 100 $\mu\text{l}$  IgG standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures total porcine fibrinogen in the 1.56-800 ng/ml range. If the unknown is thought to have high fibrinogen levels, dilutions may be made in diluent. A 1:400,000-1:2,000,000 dilution for normal porcine plasma or up to 1:10,000 dilution for normal porcine serum is suggested for best

### Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of diluent directly to the vial and agitate gently to completely dissolve contents. Add 100 $\mu\text{l}$  to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Avidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2 $\mu\text{l}$  of conjugated antibody in 10ml of diluent to generate a 1:5,000 dilution. Add 100 $\mu\text{l}$  of the 1:5,000 dilution to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Substrate Incubation

Add 100 $\mu\text{l}$  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 $\mu\text{l}$  of 1N  $\text{H}_2\text{SO}_4$  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 by gently shaking the plate.

### Measurement

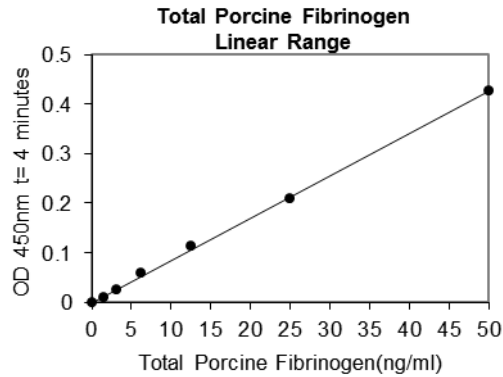
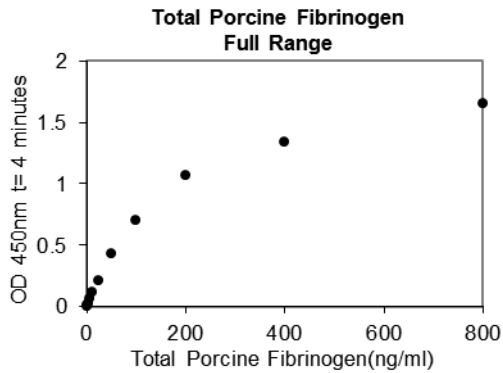
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance ( $A_{450}$ ).

### Calculation of Results

Plot  $A_{450}$  against the amount of fibrinogen in the standards. Fit a straight line through the linear points of the standard curve 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 IgG in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

results.

A typical standard curve (EXAMPLE ONLY):



## EXPECTED VALUES

The concentration of fibrinogen in normal porcine plasma ranges from 2 to 5 mg/ml and varies by strain and diet.

## PERFORMANCE CHARACTERISTICS

**Sensitivity:** The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicated (range OD450: 0.07-0.09) and calculating the corresponding concentration. The MDD was 1.25 ng/ml/

**Intra-assay Precision:** These studies are currently in progress. Please contact us for more information.

**Inter-assay Precision:** These studies are currently in progress. Please contact us for more information.

**Recovery:** These studies are currently in progress. Please contact us for more information.

**Linearity:** These studies are currently in progress. Please contact us for more information.

**Specificity:** These studies are currently in progress. Please contact us for more information.

**Sample Values:** These studies are currently in progress. Please contact us for more information.

Sample Type	Dilution	Mean (mg/mL)
Citrate Plasma	1:2,000,000	5.34
	1:1,000,000	5.44
	1:400,000	5.32
Serum	1:10,000	0.05

## 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 of or contact with the above product.







**Important Note:** During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

**Warning:** This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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