Pig Fibrinogen ELISA

For the quantitative determination of Fibrinogen in pig biological samples

Cat. No. KT-484

For Research Use Only.

PRODUCT INFORMATION

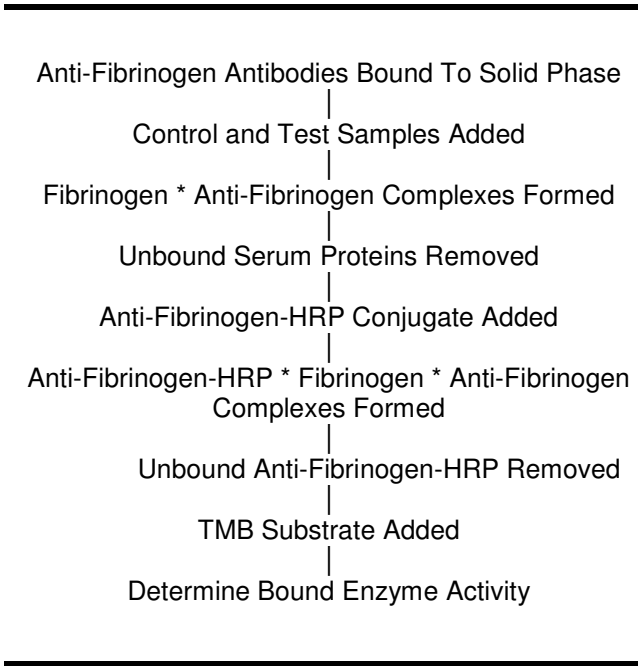
Pig Fibrinogen ELISA
Cat. No. KT-484

INTENDED USE
The Pig Fibrinogen ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the quantitative determination of Fibrinogen in pig biological samples. For research use only.

INTRODUCTION
Soluble Fibrinogen (FIB) circulates in the blood and provides the material from which the insoluble fibrin clot is formed during blood coagulation. Fibrinogen is an acute phase reactant that may be a useful marker for infection and inflammation. This ELISA kit can be used to measure Fibrinogen in biological samples of pigs.

PRINCIPLE
The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the Fibrinogen present in serum sample reacts with the anti-Fibrinogen antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound serum proteins by washing, anti-Fibrinogen antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound serum Fibrinogen. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of Fibrinogen in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of Fibrinogen in the test sample. The quantity of Fibrinogen in the test sample can be interpolated from the calibration curve constructed from the calibrators, and corrected for sample dilution.

Figure 1.



COMPONENTS

1. Diluent Concentrate
   One bottle containing 50 mL of a 5X concentrated phosphate buffered saline (PBS) solution containing 0.25% Tween, protein stabilizer and 0.25% Proclin 300 as a preservative.

2. Wash Solution Concentrate
   One bottle containing 50 mL of a 20X concentrated PBS solution with 1% Tween.

3. Enzyme-Antibody Conjugate Concentrate
   One vial containing 200 µL of a 100X concentrated affinity-purified anti-pig Fibrinogen antibody conjugated with HRP in a stabilizing buffer.

4. TMB Substrate Solution
   One vial containing 12 mL of TMB and hydrogen peroxide in citric acid buffer at pH 3.3.

5. Stop Solution
   One vial containing 12 mL of 0.3 M sulfuric acid. WARNING: Avoid contact with skin.

6. Microtiter Plate
   Twelve removable eight-well strips in well holder frame. Wells are coated with affinity-purified anti-pig Fibrinogen.

7. Pig Fibrinogen Calibrator
   One vial containing a Pig Fibrinogen Calibrator.

MATERIALS REQUIRED BUT NOT PROVIDED

- Test tubes
- Precision pipette (2 µL to 200 µL) for making and dispensing dilutions.
- Microplate washer/aspirator
- Distilled or de-ionized H_2O
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Vortex mixer

PRECAUTIONS

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.
4. Preservatives
   - Diluent contains 0.25% Proclin 300 as a preservative.
5. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.
6. Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.
7. Other precautions:
   - Do not interchange kit components from different lots.
   - Do not use kit components beyond the expiration date.
   - Protect reagents from direct sunlight.
   - Do not pipette by mouth.
   - Do not eat, drink, smoke or apply cosmetics where reagents are used.
   - Avoid all contact with the reagents by using gloves.
   - Stop solution contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

REAGENT PREPARATION

1. Diluent Concentrate
   The Diluent solution supplied is a 5X concentrate and must be diluted 1:5 with distilled or de-ionized water.

2. Wash Solution Concentrate
   The Wash Solution supplied is a 20X concentrate and must be diluted 1:20 with distilled or de-ionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. Enzyme-Antibody Conjugate Concentrate
   The Enzyme-Antibody Conjugate supplied is a 100X concentrate and must be diluted 1:100. The required amount of working conjugate solution for each microtiter plate is prepared by adding 100 µL Enzyme-Antibody Conjugate to 9.9 mL of 1X Diluent. Mix uniformly, but gently. Avoid foaming.

4. TMB Substrate Solution
   Ready to use as supplied.

5. Stop Solution
   Ready to use as supplied.

6. Microtiter Plate
   Ready to use as supplied.

7. Pig Fibrinogen Calibrator
   The Pig Fibrinogen Calibrator should be aliquoted out and stored frozen. It is at a concentration of 18.42 µg/mL and needs to be diluted in 1X diluent according to the chart below for each run. Mix well between each step. Avoid foaming.

Calibrator	Concentration (ng/mL)	Calibrator Volume added to 1X Diluent →	Volume of 1X Diluent
1	400	20 µL Fibrinogen Calibrator	901 µL
2	200	0.3 mL Calibrator 1	0.3 mL
3	100	0.3 mL Calibrator 2	0.3 mL
4	50	0.3 mL Calibrator 3	0.3 mL
5	25	0.3 mL Calibrator 4	0.3 mL
6	12.5	0.3 mL Calibrator 5	0.3 mL
7	6.25	0.3 mL Calibrator 6	0.3 mL

STORAGE AND STABILITY

1. Complete Kit
   The expiration date for the kit is stated on the outer label. The recommended storage temperature is 4°C. **Note: See long-term storage recommendations below for the Pig Fibrinogen Calibrator.**

2. Diluent
   The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4°C.

3. Wash Solution
   The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (RT, 16-25°C) or at 4°C.

4. Enzyme-Antibody Conjugate
   Undiluted horseradish peroxidase anti-Fibrinogen conjugate should be stored at 4°C and diluted immediately prior to use. The working conjugate solution is stable for one day.

5. TMB Substrate Solution
   The TMB Substrate Solution should be stored at 4°C and is stable until the expiration date.

6. Stop Solution
   The Stop Solution should be stored at 4°C and is stable until the expiration date.

7. Microtiter Plate
   Anti-pig Fibrinogen coated wells are stable until the expiration date and should be stored at 4°C in the sealed foil pouch with a desiccant pack.

8. Pig Fibrinogen Calibrator
   Aliquot Pig Fibrinogen calibrator and store them frozen. For storage longer than 14 days, keep frozen until the expiration date. Storage of less than 14 days can be at 4℃. Avoid multiple freeze/thaw cycles. The working calibrator solutions are stable for up to 12 hours after preparation.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the calibrator solutions should be within 20% of the expected values.

SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture. For plasma samples, blood may be drawn into tubes containing sodium citrate. The serum or plasma should be separated from coagulated or packed cells by centrifugation. Specimens may be shipped at room temperature (RT) and then stored refrigerated at 4°C if testing is to take place within one week after collection. If testing is to take place later than one week, specimens should be stored at -20°C. Avoid repeated freezing/thawing.

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

ASSAY PROTOCOL

Dilution of Samples

Due to the high-sensitive nature of the assay, each serum or plasma sample should be diluted before use for a normal assay. A 1:500 dilution is appropriate for most serum or plasma samples. For absolute quantification of samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required.

1. To prepare a 1:500 dilution of sample, transfer 2 µL of sample to 998 µL of 1X Diluent. This gives you a 1:500 dilution. Mix thoroughly.

Procedure

Bring all reagents to RT before use.

1. Add 100 µL of 1X Diluent to each of the wells in A1 & A2. These will serve for an evaluation of the background associated with the assay.

2. Pipette 100 µL of
   Calibrator 1 (400 ng/mL) into wells B1 & B2
   Calibrator 2 (200 ng/mL) into wells C1 & C2
   Calibrator 3 (100 ng/mL) into wells D1 & D2
   Calibrator 4 (50 ng/mL) into wells E1 & E2
   Calibrator 5 (25 ng/mL) into wells F1 & F2
   Calibrator 6 (12.5 ng/mL) into wells G1 & G2
   Calibrator 7 (6.25 ng/mL) into wells H1 & H2

3. Pipette 100 µL of diluted serum sample (test sample 1) into wells A3 & A4. The next sample goes in wells B3 & B4, the next in C3 & C4 and so on.

4. Incubate the Microtiter Plate at 22°C (RT) for thirty (30 ± 2) minutes. Keep plate level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with diluted Wash Solution, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual Wash Solution. Repeat three times for a total of four washes.

7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at 22°C (RT) for thirty (30 ± 2) minutes.
8. Wash and blot the wells as described in Steps 5 and 6.
9. Pipette 100 µL of TMB Substrate Solution into each well.
10. Incubate at RT for precisely ten (10) minutes.
11. After ten (10) minutes, add 100 µL of Stop Solution to each well.
12. Determine the absorbance at 450 nm of the contents of each well. Zero the plate reader to air.

The absorbance of the final reaction mixture can be measured up to two hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.
2. Using the results observed for the calibrators construct a calibration curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.
3. Interpolate test sample values from the calibration curve. Correct for sample dilution factor to arrive at Fibrinogen concentration in original sample.

QUALITY CONTROL

In accord with good laboratory practice, the assays for specific Fibrinogen require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or de-ionized water, washing thoroughly and accuracy of reagent and sample pipetting.

FOR RESEARCH USE ONLY