

RAT FIBRINOGEN

Immunoperoxidase Assay for Determination of Fibrinogen in Rat Samples

DIRECTIONS FOR USE

Version 1.2

INTENDED USE

The Fibrinogen test kits are a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring Fibrinogen in biological samples of rats.

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.

PRINCIPLE OF THE ASSAY

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 microtitre 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 standard curve constructed from the standards, and corrected for sample dilution.

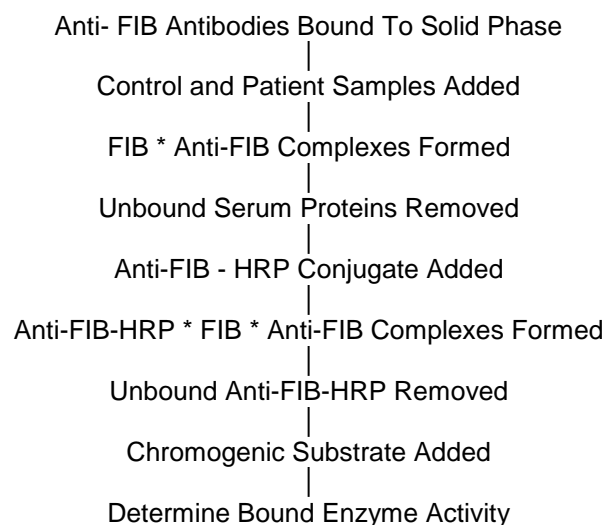


Figure 1.

REAGENTS (Quantities sufficient for 96 determinations)

1. DILUENT CONCENTRATE

One bottle containing 50 ml of a 5X concentrated phosphate buffered saline (PBS) solution containing bovine serum albumin, 0.25% Tween, and 0.25% Proclin 300 as a preservative.

2. WASH SOLUTION CONCENTRATE

One bottle containing 50 ml of a 20X concentrated phosphate buffered saline (PBS) solution containing 1% Tween.

3. 100X ENZYME-ANTIBODY CONJUGATE

One vial containing 200 μ L of affinity purified anti-Rat FIBRINOGEN antibody conjugated with horseradish peroxidase in a stabilizing buffer.

4. CHROMOGEN-SUBSTRATE SOLUTION

One vial containing 12 mL of 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

5. STOP SOLUTION

One vial containing 12 ml 0.3 M sulfuric acid.

WARNING: Avoid contact with skin.

6. Anti-Rat FIBRINOGEN COATED WELLS

Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Rat Fibrinogen.

7. Rat FIBRINOGEN Calibrator

One vial containing 0.5 ml of Rat Fibrinogen Calibrator.

FOR IN VITRO USE ONLY

REAGENT PREPARATION

1. DILUENT CONCENTRATE

The diluent supplied is a 5X Concentrate and must be diluted 1:5 with distilled or deionized water for use in this kit.

2. WASH SOLUTION CONCENTRATE

The Wash Solution supplied is a 20X Concentrate and must be diluted 1:20 with distilled or deionized 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

The required amount of working conjugate solution for each microtitre plate is prepared by adding 100 µL Enzyme-Antibody Conjugate to 10 mL of Diluent. Mix uniformly, but gently. Avoid foaming.

4. CHROMOGEN-SUBSTRATE SOLUTION

Ready to use as supplied.

5. STOP SOLUTION

Ready to use as supplied.

6. FIBRINOGEN ELISA Micro plate

Ready to use as supplied.

7. FIBRINOGEN STANDARDS

The Rat Fibrinogen Calibrator should be aliquoted out and stored frozen. It is at a concentration of 12 ug/ml and needs to be diluted in 1X diluent according to the chart below for each run. Mix well between each step. Avoid foaming. For samples containing lower levels of Fibrinogen, it is possible to extend the utility of the lower detection limit of this assay by further serial 2-fold dilution of standard # 6.

Standard	ng/ml	Amount	1X Diluent
1	800	40 µl Fibrinogen Calibrator	560 µl
2	400	0.3 ml standard 1	0.3 ml
3	200	0.3 ml standard 2	0.3 ml
4	100	0.3 ml standard 3	0.3 ml
5	50	0.3 ml standard 4	0.3 ml
6	25	0.3 ml standard 5	0.3 ml

STORAGE AND STABILITY

The expiration date for the package is stated on the box label.

1. 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 4-8°C.

2. 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 (16-25°C) or at 4-8°C.

3. ENZYME-ANTIBODY CONJUGATE

Undiluted horseradish peroxidase anti-Fibrinogen conjugate should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for one day.

4. CHROMOGEN-SUBSTRATE SOLUTION

The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.

5. STOP SOLUTION

The Stop Solution should be stored at 4-8°C and is stable until the expiration date.

6. FIBRINOGEN ELISA Micro plate

Anti-Rat Fibrinogen coated wells are stable until the expiration date, and should be stored at 4-8°C in the sealed foil pouch with desiccant pack.

7. FIBRINOGEN CALIBRATOR

Aliquot Rat 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 4°C. The calibrator is stable until the expiration date but the working standard solution is stable for up to 12 hours after preparation.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the standard 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 the coagulated or packed cells by centrifugation. Specimens may be shipped at room temperature and then stored refrigerated at 2-8°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 freeze/thawing.

1. Precautions

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

2. Additives and Preservatives

No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

3. Known interfering substances

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

MATERIAL PROVIDED

See "REAGENTS"

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (2 μ L to 200 μ L) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H₂O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Vortex mixer

ASSAY PROTOCOL

DILUTION OF SERUM SAMPLES

The assay for quantification of Fibrinogen in serum and plasma requires that each test sample be diluted before use. A 1:100 dilution is appropriate for most serum samples, and a 1:10,000 dilution is appropriate for most plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required.

1. To prepare a 1:100 dilution of sample, transfer 3 μ L to 297 μ L of diluent. This gives you a 1:100 dilution. Mix thoroughly.
2. To prepare a 1:10,000 dilution of sample, transfer 5 μ L of sample to 495 μ L of diluent. This gives you a 1:100 dilution. Next, add 5 μ L of your 1:100 diluted sample to 495 μ L of diluent. You now have a 1:10,000 dilution of your sample. Mix thoroughly at each stage.

PROCEDURE

Bring all reagents to room temperature before use.

1. Add 100 μ L of Diluent to each of the wells in 1A & 2A. These will serve for an evaluation of the background associated with the assay.
2. Pipette 100 μ L of
 - Standard 1 (800 ng/ml) into wells 1B & 2B
 - Standard 2 (400 ng/ml) into wells 1C & 2C
 - Standard 3 (200 ng/ml) into wells 1D & 2D
 - Standard 4 (100 ng/ml) into wells 1E & 2E
 - Standard 5 (50 ng/ml) into wells 1F & 2F
 - Standard 6 (25ng/ml) into wells 1G & 2G
3. Pipette 100 μ L of serum sample (test sample 1) into wells 1H & 2H. The next sample goes in wells 3A & 4A, the next in 3B & 4B and so on.
4. Incubate the micro titer plate at 22°C (room temperature) for sixty (60 \pm 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 wash buffer, 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 buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 μL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at 22°C (room temperature) for thirty (30 \pm 2) minutes.

8. Wash and blot the wells as described in Step 5/6.

9. Pipette 100 μL of TMB Substrate Solution into each well.

10. Incubate at room temperature for precisely ten (10) minutes.

11. After ten (10) minutes, add 100 μL of Stop Solution to each well.

12. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to air.

STABILITY OF THE FINAL REACTION MIXTURE

The absorbance of the final reaction mixture can be measured up to 2 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 standards construct a Standard 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 standard curve. Correct for sample dilution factor to arrive at the 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 deionized water, washing thoroughly and accuracy of reagent and sample pipettings.