# RAT FIBRINOGEN ELISA
# Life Diagnostics, Inc., Catalog Number: FIB-2

## INTRODUCTION

Fibrinogen is dimeric protein (mwt, 340 kDa), that is synthesized in the liver and circulates in rat plasma at a concentration of approximately 3 mg/ml. It is significantly elevated during the acute phase response[1,2] and therefore serves as a useful marker of infection, disease, and inflammation.

## PRINCIPLE OF THE ASSAY

The assay uses affinity purified rat fibrinogen antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated rat fibrinogen antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 30 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 30 minutes. This results in fibrinogen molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If fibrinogen is present a blue color develops. Color development is stopped by the addition of Stop Solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of fibrinogen is proportional to absorbance and is derived from a standard curve.

## MATERIALS AND COMPONENTS

**Materials provided with the kit:**

- Fibrinogen antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- Fibrinogen stock
- 20x Wash Solution: TBS50-20, 50 ml
- 10x Diluent: YD25-10, 25 ml
- TMB: TMB11-1, 11 ml
- Stop Solution: SS11-1, 11 ml

**Materials required but not provided:**

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

## STORAGE

The kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. The kit will remain stable for six months from the date of purchase.

## GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

## DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one volume of the 10x stock with nine volumes of distilled or deionized water.

## WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

## STANDARD PREPARATION

1. The rat fibrinogen stock is provided lyophilized. Add the volume of distilled or deionized water indicated on the vial label and mix gently until dissolved to obtain a 2 μg/ml stock ***(the reconstituted standard should be aliquoted and frozen at -20°C after reconstitution if additional use is intended)***.
2. Label 7 polypropylene or glass tubes as 250, 125, 62.5, 31.2, 15.6, 7.8 and 3.9 ng/ml.
3. Dispense 525 μl of diluent into the tube labeled 250 ng/ml and 300 μl of diluent into the remaining tubes.
4. Pipette 75 μl of the 2 μg/ml fibrinogen standard into the tube labeled 250 ng/ml and mix. This provides the working 250 ng/ml fibrinogen standard.
5. Prepare a 125 ng/ml standard by diluting and mixing 300 μl of the 250 ng/ml standard with 300 μl of diluent in the tube labeled 125 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

## SAMPLE PREPARATION

Fibrinogen is present in normal rat plasma at a concentration of ~ 3 mg/ml. To obtain values within the range of the standard curve we suggest that samples be diluted 100,000-fold using the following procedure for each sample to be tested.

1. Dispense 998 μl and 497.5 μl of 1x diluent into separate tubes.
2. Pipette and mix 2 μl of the plasma sample into the tube containing 998 μl of diluent. This provides a 500-fold dilution.
3. Mix 2.5 μl of the 500-fold diluted sample with the 497.5 μl of 1x diluent in the second tube. This provides a 100,000-fold dilution.

## ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 μl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 30 minutes.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 μl/well).

5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µl of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 30 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µl of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 µl of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

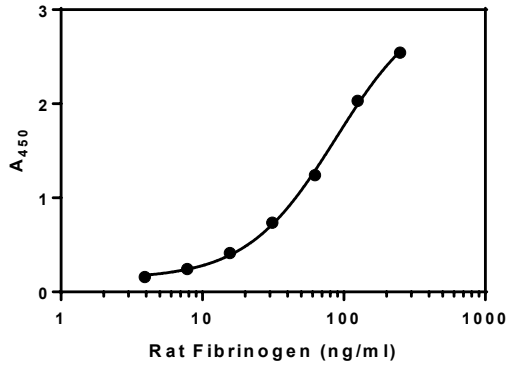
## CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus $\log_{10}$ of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the standard curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the $A_{450}$ values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

## TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns.

| Fibrinogen (ng/ml) | Absorbance (450 nm) |
|---|---|
| 250.0 | 2.543 |
| 125.0 | 2.031 |
| 62.5 | 1.240 |
| 31.25 | 0.735 |
| 15.63 | 0.413 |
| 7.81 | 0.244 |
| 3.91 | 0.159 |




## REFERENCES

1. G Schreiber et. al., The acute phase response in the rodent. Ann N Y Acad Sci. 557:61-85 (1989)
2. A Larrson, J Bjork and C Lundberg. Nephelometric Determination of rat fibrinogen as a marker of inflammatory response. Vet Immunol Immunopathol 59:163-169 (1997)

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