KAMIYA BIOMEDICAL COMPANY

Rat Fibrinogen ELISA

For the quantitative determination of fibrinogen in rat plasma

Cat. No. KT-1895

For Research Use Only.

PRODUCT INFORMATION
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PRODUCT
The **K-ASSAY®** Rat Fibrinogen ELISA is an enzyme immunoassay for the quantitative determination of fibrinogen in rat plasma. For research use only.

INTRODUCTION
Fibrinogen is a 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 and therefore serves as a useful marker of infection, disease and inflammation.

PRINCIPLE
The assay uses affinity purified rat fibrinogen antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated rat fibrinogen antibodies for detection. Calibrators 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, and absorbance is measured at 450 nm. The concentration of fibrinogen is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
- Fibrinogen antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 mL
- Fibrinogen calibrator stock
- 20x Wash solution, 50 mL
- 10x Diluent, 25 mL
- TMB, 11 mL
- Stop solution, 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℃ and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date shown on the box label.

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. The assay was calibrated using a shaking incubator set at 150 rpm and 25℃. Performance of the assay at lower temperatures and mixing speeds may result in lower absorbance

values.

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 de-ionized water.

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.

CALIBRATOR PREPARATION

1. The rat fibrinogen calibrator 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 calibrator 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 calibrator into the tube labeled 250 ng/mL and mix. This provides the working 250 ng/mL fibrinogen calibrator.
5. Prepare a 125 ng/mL calibrator by diluting and mixing 300 µL of the 250 ng/mL calibrator with 300 µL of diluent in the tube labeled 125 ng/mL.
6. Similarly prepare the remaining calibrators 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 calibration 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 calibrators and samples into the wells (we recommend that calibrators 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.

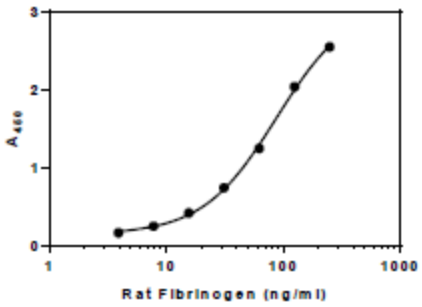
CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus $\log_{10}$ of the concentration.
2. Fit the calibration curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the calibration 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 calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration 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



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KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com