Catalog # RFBGNKT

Rat Fibrinogen Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This rat fibrinogen antigen assay is intended for the quantitative determination of total fibrinogen antigen in rat plasma and serum.

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

Rat fibrinogen will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled polyclonal anti-rat fibrinogen primary antibody binds to the captured protein. Excess antibody is washed away and bound polyclonal antibody is reacted with avidin 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 rat fibrinogen. Color development is proportional to the concentration of fibrinogen in the samples.

REAGENTS PROVIDED

- **96-well microtiter strip plate (8X12 removable wells):**
  Fibrinogen capture antibody coated
- **5X Diluent:**
  1 bottle of 50ml; bring to 1X using DI water
- **10X Wash Buffer:**
  1 bottle of 50ml; bring to 1X using DI water
- **Rat fibrinogen antigen standard:**
  1 vial of lyophilized standard
- **Anti-rat fibrinogen primary antibody:**
  1 vial of lyophilized biotin labeled polyclonal antibody
- **Avidin peroxidase secondary reagent:**
  1 vial of concentrated HRP labeled avidin
- **TMB substrate solution:**
  1 bottle of 10ml solution
- **Stop solution:**
  1 bottle of 6ml 1M sulfuric acid

STORAGE AND STABILITY

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes, 1.5ml centrifuge tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm. (OPTIONAL)

WARNINGS

Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

PRECAUTIONS

- **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.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **Diluent concentrate:** The diluent supplied in a 5X concentrate and must be diluted 1:5 with deionized water for use with the kit.
- **Wash buffer concentrate:** The wash buffer supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

SPECIMEN COLLECTION

The assay measures total rat fibrinogen in the 3.125-800 ng/ml range. Samples giving rat fibrinogen levels above 800ng/ml should be diluted in 1X diluent before use. Normal plasma samples need to be diluted between 1:10,000 and 1:50,000 in 1X diluent for the values to be within linear range of the standard curve.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay. If a microtiter plate shaker is not available then keep the plate on a flat surface for 60 minutes at each step instead of 30 minutes.
Note: when the assay is performed without shaking the plate, the final absorbance values at 450nm will be lower than when the assay is performed using a plate shaker.

Preparation of Standard:
Reconstitute standard vial with 5 ml of 1X diluent to give a 800ng/ml solution.
Dilution table for preparation of rat fibrinogen standards:

Fibrinogen concentration (ng/ml)	Dilutions
800	Straight from the vial
400	500µl (1X Diluent) + 500µl (800ng/ml)
200	500µl (1X Diluent) + 500µl (400ng/ml)
100	500µl (1X Diluent) + 500µl (200ng/ml)
50	500µl (1X Diluent) + 500µl (100ng/ml)
25	500µl (1X Diluent) + 500µl (50ng/ml)
12.5	500µl (1X Diluent) + 500µl (25ng/ml)
6.25	500µl (1X Diluent) + 500µl (12.5ng/ml)
3.125	500µl (1X Diluent) + 500µl (6.25ng/ml)
0	500µl (1X 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. Add 100µl 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 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Primary Antibody Addition:
Add 10ml of 1X diluent directly to the primary antibody vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

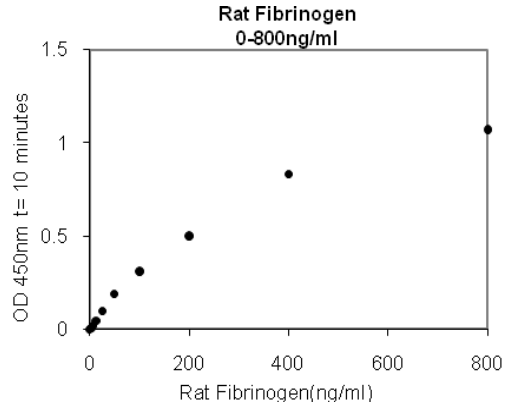
Secondary Reagent Addition:
Dilute 2.5µl into 10ml of 1X diluent and mix well and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

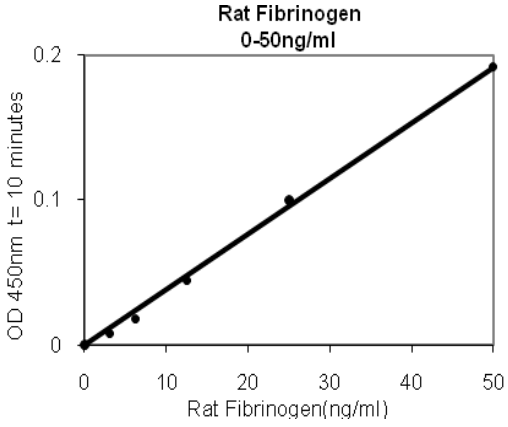
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 10-20 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance values at 450nm. For best results read plate immediately.

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

Assay Calibration:
Plot A_{450} against the amount of rat fibrinogen in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total rat fibrinogen in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





Example of the plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	3.125 ng/ml	6.25 ng/ml	12.5 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	400 ng/ml	800 ng/ml		
B	0	3.125 ng/ml	6.25 ng/ml	12.5 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	400 ng/ml	800 ng/ml		
C												
D												
E												
F												
G												
H												

96 well plate
Standards: 20 wells
Samples: 76 wells

EXPECTED VALUES

Fibrinogen is present in normal rat plasma at a concentration of 3.1 mg/ml [5] and varies by age and diet [6]. Normal plasma samples need to be diluted between 1:10,000 and 1:50,000 in 1XDiluent for the values to be within linear range of the standard curve.

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 or from contact with the above product.

REFERENCE

1. Kamath S and Lip GYH. Fibrinogen: biochemistry, epidemiology and determinants. QJM 2003; 96: 711-729.
2. Kusher I. The phenomenon of acute phase response. Ann New York Acad Sci 1982; 389: 39-48.
3. Kannel WB et al. Fibrinogen and risk of cardiovascular disease. The Framingham Study. J Am Med Assoc 1987;258:1183-1186.
4. Handa K et al. Plasma fibrinogen levels an independent indicator of severity of coronary atherosclerosis. Atherosclerosis 1989;77:209-213.
5. Larrson A et al. Nephelometric Determination of rat fibrinogen as a marker of inflammatory response. Vet Immunol Immunopathol 1997; 59:163-169.
6. Dorner H et al. Influence of age, treadmill running, and food restriction on plasma fibrinogen concentration and expression of gamma chain mRNA in female Sprague-Dawley rats. Gerontology 1995; 41:252-259.