

# FIBROTEK FIB

Fibrinogen Assay Kit. 100 Determinations



## INTENDED USE

The **FIBROTEK FIB** Fibrinogen Assay Kit is intended for use in the quantitative determination of fibrinogen in citrated human plasma.

## SUMMARY

Fibrinogen, a high molecular weight glycoprotein synthesized in the liver, plays a fundamental role in hemostasis. The interaction between thrombin and fibrinogen leads to production of the insoluble cross-linked polymer fibrin. For normal hemostasis to occur in response to injury or tissue damage, a sufficient concentration of fibrinogen must be present in plasma. Quantitation of plasma fibrinogen can be important in disease states such as disseminated intravascular coagulation (DIC), liver disease, and thrombolytic therapy. Rare congenital deficiencies of fibrinogen can occur including afibrinogenemia and hypofibrinogenemia. Dysfibrinogenemias in which abnormal molecular forms of fibrinogen are present can also occur. Elevated levels of fibrinogen can be found in acute phase reactant responses, pregnancy, and oral contraceptive use.

## PRINCIPLE

Quantitative measurement of fibrinogen is most commonly done using the Clauss technique, which involves measuring the clotting time of dilute plasma after the addition of thrombin. At high thrombin concentrations (>30 NIH units/mL) and low fibrinogen concentrations, the fibrinogen level is inversely proportional to the thrombin clotting time plotted on log - log graph paper

## REAGENTS

**Warning: FOR *IN-VITRO* DIAGNOSTIC USE ONLY.**

### 1. Thrombin Reagent

**Ingredients:** The reagent contains a lyophilized preparation of human thrombin of approximately 100 NIH units/mL plus added stabilizers.

**Preparation for use:** Reconstitute each vial of thrombin reagent with 2.0 mL of distilled water as indicated on the vial label. Invert gently to mix, do not shake, and allow to stand for 10 min. at room temperature before use.

**Storage and stability:** The lyophilized product should be stored at 2-8°C until the expiration date on the vial. After reconstitution, the thrombin solution is stable for 8 hours at room temperature (20-24°C) or 1 week at 2-8°C. Do not use if precipitation occurs during storage.

### 2. Fibrinogen Calibrator

**Ingredients:** The calibrator is lyophilized normal human plasma assayed for fibrinogen by a functional clotting assay. See vial label for assigned assay value for the current lot given in mg/dL.

**Preparation for use:** Reconstitute each vial with 1 mL of distilled water. Swirl gently to mix; do not shake. Allow to stand for 15 minutes at room temperature (20-24°C) before use.

**WARNING: POTENTIAL BIOHAZARD.** The plasma used to prepare the fibrinogen calibrator has been tested and found negative for Hepatitis B antigen (HBsAg) and antibodies to HIV and HCV by FDA licensed tests.

However the calibrator should be handled with the same precautions as those observed when handling potentially infectious patient plasmas.

**Storage and stability:** The reagent is stable until the date indicated on the label when stored at 2-8°C. After reconstitution, the calibrator is stable for 8 hours at 2-8°C.

### 3. Imidazole Buffer

**Ingredients:** Buffer contains 15 mM Imidazole, 0.125 M Sodium Chloride with 0.02% sodium azide as preservative.

**WARNING: Sodium Azide.** The Imidazole buffer is preserved with sodium azide, which can form highly explosive metal azides if exposed to lead or copper in plumbing. Any such materials should be discarded into a sink only with large volumes of water to minimize such a risk.

**Preparation for use:** The buffer is packaged ready for use.

**Storage and stability:** The buffer is stable until the date indicated on the label when stored at 2-8°C.

## TECHNIQUES

The fibrinogen assay may be performed by accepted manual methods, or by using optical or electromechanical coagulation analyzers.

## SPECIMEN COLLECTION AND PREPARATION

**Specimen:** Plasma obtained from whole blood anticoagulated with 0.1M sodium citrate.

**Specimen Collection:** Nine parts freshly collected whole blood should be immediately added to one part citrate anticoagulant and mixed thoroughly.

**Specimen Preparation:** Centrifuge the whole blood at 2500 x g for 15 minutes (NCCLS H21-A2, 1991). Immediately separate the plasma from the red cells using a plastic pipette (if necessary), and place in a plastic test tube.

**Storage and stability:** Before and during testing, the samples must be tested within 2 hours if stored at 22-24°C. Frozen samples should be thawed rapidly at 37°C before testing (NCCLS H21-A2).

## TEST PROCEDURE

### Materials provided:

100 determinations  
5 vials Thrombin reagent-2 mL  
3 vials Fibrinogen Calibrator -1 mL  
1 bottle Imidazole buffer -135 mL  
Instructions for Use

### Materials required but not provided:

Pipettes for 50, 100 and 200µL volumes  
12 x 75 mm plastic test tubes  
Stopwatch or timing device  
Coagulation analyzer  
37°C waterbath or heating block  
Instrument cuvettes  
log paper

### Additional equipment and supplies available from r<sup>2</sup> Diagnostics:

PlasmaCon N (Normal Control Plasma)  
PlasmaCon L-1 (Abnormal Control Plasma)  
PlasmaConL-2 (Abnormal Control Plasma)

## STEP-BY-STEP METHOD

The following is the manual method. Please refer to the User Manual for instructions, if an automated instrument is to be used.

### A. Specimen and Reagent Preparation

1. All test tubes, syringes and pipettes should be plastic
2. Collect and prepare the blood sample specimen according to the directions outlined in the **SPECIMEN COLLECTION AND PREPARATION** section.
3. Prepare the reagents according to the reconstitution instructions in the **REAGENTS** section.

### B. Preparation of Fibrinogen Reference Curve

1. Allow all reagents to equilibrate to room temperature.
2. Using Imidazole Buffer, prepare dilutions of Fibrinogen Calibrator: 1:5, 1:10, 1:20, 1:30 and 1:40 in 12 x 75mm test tubes as follows

	1:5	1:10	1:20	1:30	1:40
Buffer	0.8mL	0.9mL	1.9mL	2.9mL	3.9mL
Calibrator	0.2mL	0.1mL	0.1mL	0.1mL	0.1mL

3. Perform duplicate determinations on each dilution of the Fibrinogen Calibrator as follows:
  - (a) Pipette 200µL of diluted calibrator into a test tube and incubate for 2 minutes at 37° C.
  - (b) Add 100µL of Thrombin Reagent and immediately start the timing device.
  - (c) Obtain the clotting times for each of the dilutions of the Fibrinogen Calibrator.

### C. Testing of Patient Specimen

1. Dilute the test plasma 1:10 in Imidazole buffer
2. Pipette 200µL of test plasma into test tube and incubate for 2 minutes at 37° C.
3. Add 100µL of Thrombin Reagent and immediately start the timing device.
4. Record the clotting time and average the duplicates to obtain the mean value.
5. Obtain the mean clotting times for each sample of the test plasma.

### Quality Control

Quality control of assays involves multiple components. Each laboratory should establish a quality control program that includes normal and abnormal controls plasmas. [PlasmaCon N](#), [PlasmaCon L-1](#) and [PlasmaCon L-2](#) have been assayed for fibrinogen and are recommended for use. If the controls do not perform within the reference range, patient results should be considered invalid and not reported.

## RESULTS

### Standard Curve

1. Use log-log graph paper or spreadsheet software to construct the reference standard curve.
2. Plot the mean clotting time for each dilution of the Fibrinogen Calibrator on the Y-axis and the concentration of each dilution on the X-axis. Construct a best-fit straight line using all 5 points.

### Test Plasma

1. Plot the mean clotting time of the 1:10 dilution on the reference curve.
2. Interpolate the result by drawing a straight line from the clotting time point down through the X axis to give the fibrinogen concentration in mg/dL.
3. For plasmas with dilutions of other than 1:10 i.e. 1:20, the concentration read from the curve must be multiplied by the dilution factor. If a dilution of 1:20 was used, then the result must be multiplied by 2 to compensate for the dilution.

## LIMITATIONS

Significant levels of heparin and elevated levels of fibrin(ogen) degradation products (FDP) in the patient plasma can cause falsely low fibrinogen results. However because of the high thrombin concentration used in this kit, therapeutic plasma heparin levels do not interfere.

## PERFORMANCE CHARACTERISTICS

### 1. Precision

Precision studies were performed to establish Within Run and Between Run CV's for normal controls and abnormal controls. Assays were performed using photo-optical and mechanical coagulation analyzers.

<i>Normal</i>	<i>Within Run</i>	<i>Between Run</i>
<b>n</b>	<b>40</b>	<b>20</b>
<b>Mean</b>	<b>272.7 mg/dL</b>	<b>275.8 mg/dL</b>
<b>SD</b>	<b>14.1 mg/dL</b>	<b>9.69 mg/dL</b>
<b>CV</b>	<b>3.55%</b>	<b>3.43%</b>

<i>Abnormal</i>		
<b>n</b>	<b>40</b>	<b>20</b>
<b>Mean</b>	<b>168.0 mg/dL</b>	<b>162.7 mg/dL</b>
<b>SD</b>	<b>5.4 mg/dL</b>	<b>8.02 mg/dL</b>
<b>CV</b>	<b>3.2%</b>	<b>4.81%</b>

### 2. Comparison

A comparison study was done using the FibroTek assay and a comparative method on 110 normal and abnormal samples using two different coagulation analyzer types. The linear regression equations and the coefficient of determination ( $r^2$ ) were as follows.

Photo-optical  
**n=110**      **Y = 0.8592x + 8.565**      **r<sup>2</sup> = 0.9693**

Mechanical  
**n=110**      **Y = 0.8479x + 21.941**      **r<sup>2</sup> = 0.9582**

Y = FibroTek FIB kit  
X = Reference Kit

