

FIBROTEK FIB

Fibrinogen Assay Kit. 100 Determinations



C. Testing of Patient Specimen

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

- Use log-log graph paper or spreadsheet software to construct the reference standard curve.
- 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

- Plot the mean clotting time of the 1:10 dilution on the reference curve.
- 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.
- 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>
n	40	20
Mean	272.7 mg/dL	275.8 mg/dL
SD	14.1 mg/dL	9.69 mg/dL
CV	3.55%	3.43%

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

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 (r²) were as follows.

Photo-optical n =110	Y = 0.8592x + 8.565	r² = 0.9693
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Mechanical n=110	Y = 0.8479x + 21.941	r² = 0.9582
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Y = FibroTek FIB kit
X = Reference Kit

Version Française

APPLICATION

Le kit de dosage du fibrinogène **FIBROTEK FIB** est conçu pour être utilisé dans la détermination quantitative du fibrinogène dans du plasma humain citraté.

RÉSUMÉ

Le fibrinogène est une glycoprotéine de poids moléculaire élevé synthétisée dans le foie, qui joue un rôle fondamental dans l'hémostase. L'interaction entre la thrombine et le fibrinogène entraîne la production de fibrine, un réseau de polymères insolubles entrecroisés. Pour que l'hémostase ait lieu normalement en cas de blessure ou de lésion tissulaire, il faut une concentration suffisante en fibrinogène dans le plasma. La quantification du fibrinogène présent dans le plasma peut être importante pour le diagnostic de certaines pathologies telles la coagulation intravasculaire disséminée (CIVD) ou les maladies hépatiques, ainsi que pour l'établissement d'un traitement thrombolytique. Les déficiences congénitales en fibrinogène sont rares, parmi lesquelles l'afibrinogénémie et l'hypofibrinogénémie. Ce dosage est également important pour identifier les dysfibrinogémies, où le fibrinogène est présent mais comporte des anomalies moléculaires. Le taux de fibrinogène augmente dans les réactions de phase aiguë, lors de la grossesse et de la prise de contraceptifs oraux.

PRINCIPE

La mesure quantitative du fibrinogène fait le plus souvent appel à la technique de Clauss, qui mesure le temps de coagulation du plasma dilué après ajout de thrombine. Lorsque la concentration en thrombine est élevée (> 30 unités NIH/ml) et la concentration en fibrinogène faible, le taux de fibrinogène est inversement proportionnel au temps de coagulation de la thrombine apparaissant sur le graphique logarithmique.

RÉACTIFS

Avertissement : POUR UTILISATION DIAGNOSTIQUE *IN VITRO* UNIQUEMENT

- Réactif thrombine**

Ingrédients: Le réactif contient une préparation lyophilisée de thrombine humaine d'environ 100 unités NIH/ml, ainsi que des stabilisateurs ajoutés.

Préparation avant utilisation : Reconstituer chaque flacon de réactif thrombine avec 2,0 ml d'eau distillée comme indiqué sur l'étiquette du flacon. Retourner doucement le flacon pour mélanger, sans secouer, et laisser reposer pendant 10 minutes à température ambiante avant utilisation.

Conservation et stabilité : Le produit lyophilisé doit être conservé entre 2 et 8 °C jusqu'à la date d'expiration indiquée sur le flacon. Après reconstitution, la solution de thrombine est stable pendant 8 heures si elle est conservée à température ambiante (20-24 °C) ou pendant une semaine si elle est conservée entre 2 et 8°C. Ne pas utiliser si un précipité s'est formé au cours de la conservation.

2. Calibrateur fibrinogène

Ingrédients: Le calibrateur est du plasma humain normal lyophilisé dont le fibrinogène a été dosé par un test fonctionnel de coagulation. Se reporter à l'étiquette du flacon pour connaître la valeur attribuée pour le lot (en mg/dl).

Préparation avant utilisation : Reconstituer chaque flacon avec 1 ml d'eau distillée. Remuer doucement pour mélanger ; ne pas secouer. Laisser reposer pendant 15 minutes à température ambiante (20-24 °C) avant utilisation.

AVERTISSEMENT : RISQUE BIOLOGIQUE : Le plasma utilisé pour préparer le calibrateur fibrinogène s'est avéré négatif à l'antigène de l'hépatite B (HBsAg) et aux anticorps anti-VHC et anti-VIH lors de l'utilisation de tests agréés par la FDA.

Cependant, le calibrateur doit être manipulé avec les mêmes précautions que celles observées lors de la manipulation de plasmas de patients potentiellement infectieux.

English Version

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

Ingrédients: 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 r² 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

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

B. Preparation of Fibrinogen Reference Curve

- Allow all reagents to equilibrate to room temperature.
 - 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 |

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

B. Preparación de la curva de referencia de fibrinógeno

1. Deje que todos los reactivos se equilibren a temperatura ambiente.
2. Utilizando el tampón de imidazol, prepare las diluciones de calibrador de fibrinógeno: 1:5, 1:10, 1:20, 1:30 y 1:40 en tubos de ensayo 12 x 75mm de la siguiente manera:

	1:5	1:10	1:20	1:30	1:40
Tampón	0,8 mL	0,9 mL	1,9 mL	2,9 mL	3,9 mL
Calibrador	0,2 mL	0,1 mL	0,1 mL	0,1 mL	0,1 mL

3. Realice determinaciones por duplicado en cada dilución del calibrador de fibrinógeno de la siguiente manera:
(a) Pipetee 200 µL de calibrador diluido en un tubo de ensayo e incube durante 2 minutos a 37 °C.
(b) Añada 100 µL de reactivo de trombina e inicie inmediatamente el temporizador.
(c) Obtenga los tiempos de coagulación de cada una de la diluciones del calibrador de fibrinógeno.

C. Pruebas de muestras de pacientes

1. Diluya el plasma de la prueba con una relación de 1:10 en el tampón de imidazol
2. Pipetee 200 µL del plasma de la prueba en un tubo de ensayo e incube durante 2 minutos a 37 °C.
3. Añada 100 µL de reactivo de trombina e inicie inmediatamente el temporizador.
4. Registre el tiempo de coagulación y calcule el promedio de los duplicados para obtener el valor de media.
5. Obtenga los tiempos de coagulación medios de cada muestra del plasma de la prueba.

Control de calidad

El control de calidad de los ensayos implica varios aspectos. Cada laboratorio debe fijar un programa de control de calidad que incluya plasma de control normal y anormal. **PlasmaCon N**, **PlasmaCon L-1** y **PlasmaCon L-2** se han ensayado para el fibrinógeno y su uso está recomendado. Si el resultado de los controles no está dentro de su intervalo de referencia, los resultados del paciente deben considerarse no válidos y no deben registrarse.

RESULTADOS

Curva estándar

1. Utilice papel doblemente logarítmico o software de hoja de cálculo para crear la curva estándar de referencia.
2. Trace el tiempo de coagulación medio de cada dilución del calibrador de fibrinógeno en el eje Y y la concentración de cada dilución en el eje X. Cree una línea recta de ajuste óptimo con los 5 puntos.

Plasma de la prueba

1. Trace el tiempo de coagulación medio de la dilución con una relación de 1:10 en la curva de referencia.
2. Interpole el resultado dibujando una línea recta descendente desde el punto del tiempo de coagulación a través del eje X para obtener la concentración de fibrinógeno en mg/dL.
3. Para plasma con diluciones distintas de 1:10, como por ejemplo 1:20, la lectura de la concentración desde la curva se debe multiplicar por el factor de dilución. Si se ha utilizado una dilución de 1:20, el resultado se debe multiplicar por 2 para compensar la dilución.

LÍMITES

Los niveles significativos de heparina y los elevados niveles de productos de degradación de la fibrina (fibrinógeno) (FDP) en el plasma de paciente pueden originar falsos resultados de fibrinógeno bajo. Sin embargo, debido a la alta concentración de trombina utilizada en este kit, los niveles de heparina del plasma terapéutico no producen interferencias.

CARACTERÍSTICAS DE RENDIMIENTO

1. Precisión

Se realizaron estudios de precisión para establecer los CV intraanálisis y entre análisis para los controles normales y anormales. Los ensayos se llevaron a cabo mediante analizadores de coagulación fotoópticos y mecánicos.

<i>Normal</i>	<i>Intraanálisis</i>	<i>Entre análisis</i>
n	40	20
Media	272,7 mg/dL	275,8 mg/dL
DT	14,1 mg/dL	9,69 mg/dL
CV	3,55%	3,43%

<i>Anormal</i>		
n	40	20
Media	168,0 mg/dL	162,7 mg/dL
DT	5,4 mg/dL	8,02 mg/dL
CV	3,2%	4,81%

2. Comparación

Se realizó un estudio comparativo mediante el ensayo FibroTek y se utilizó un método comparativo sobre 110 muestras normales y anormales utilizando dos tipos distintos de analizadores de coagulación. Las ecuaciones de la regresión lineal y el coeficiente de determinación (r²) fueron los siguientes:

Fotoóptico		
n = 110	Y = 0,8592x + 8,565	r² = 0,9693
Mecánico		
n = 110	Y = 0,8479x + 21,941	r² = 0,9582

Y = kit FibroTek FIB

X = kit de referencia



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