

# Fibrinogen

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<b>REF</b>	3510301	Fibrinogen	5 x 2 mL
For <i>in vitro</i> diagnostic use only			

## Fibrinogen

### CLAUSS METHOD

Determination of fibrinogen time

### PRINCIPLE

The Clauss method measures the rate of fibrinogen to fibrin conversion in the presence of excess of thrombin and has been shown to be rapid, sensitive and precise.

When diluted plasma is clotted with excess of thrombin, the fibrinogen level is inversely proportional to the clotting time.

### REAGENT COMPOSITION

**Fibrinogen** Bovine thrombin,  $\approx$  100 NIH U/mL in a buffer, stabilizers and preservative.

**Imidazole** Imidazole buffer, stabilizers and preservative.


Optative. **Plasma Control Level 1**

Ref. 3520101

**Plasma Control Level 2**

Ref. 3520201.

### STORAGE AND STABILITY

 Store at 2-8°C. The reagents are stable until the expiry date stated on the label, when stored in the original container.

The reconstituted reagent is stable 7 days when stored in the original container at 2-8°C.

Do not freeze.

### REAGENT PREPARATION

1. Reconstitute the contents of Fibrinogen with **2.0 mL** of detolled water.
2. Replace the stopper and thoroughly mix the vial contents. Let stand for no less than 30 minutes prior to use to assure complete hydration of the contents.

### SAMPLES

Test plasma should be prepared from citrated whole blood **without** heparin, EDTA or oxalate.

#### 1. Blood Collection

- Draw venous blood into a plastic or siliconized **syringe**. Immediately transfer 9.0 mL of blood into a tube containing 1.0 mL of 3.2% or 3.8% sodium citrate solution.
- or
- Draw venous blood into a commercial **vacuum tube** containing 3.2% or 3.8% sodium citrate solution.
- Insure that a full draw has been obtained since the ratio of 9 parts blood to 1 part citrate is critical. A heparinized lock or transfer line should not be used. It is generally recommended that the second or third tube draw be used for coagulation tests.

#### 2. Plasma Preparation

- Mix well by inversion and centrifuge at 2,500 x g for 15 minutes soon after blood collection. Unless samples are to be processed immediately, transfer the plasma into a plastic tube. Plasma that is clearly hemolyzed or contains > 10,000 platelets per mL or red cells is not suitable for coagulation testing.

#### 3. Plasma Storage

- Plasma samples may be stored at room temperature (18 to 26°C) for up to 2 hours; refrigerated (2 to 8°C) for up to 4 hours; frozen at -20°C for up to 2 months or at -70°C for up to 6 months. Plasma may be re-centrifuged prior to freezing to assure that all cells are removed. Quick thaw frozen samples and test them immediately. The samples should not have any contact with glass.

### INTERFERENCES

- Fibrinogen clotting times may be prolonged by substances including corticosteroids, EDTA, oral contraceptives, asparaginase, clofibrate, erythromycin, ethanol, tetracycline and anticoagulants such as heparin and Coumadin.
- Fibrinogen may be shortened by substances including antihistamines, butabarbital, caffeine, oral contraceptives, phenobarbital and vitamin K<sup>10</sup>.

### ADDITIONAL EQUIPMENT

- Coagulometer or stopwatch and bath at 37°C  $\pm$  0.5°C.
- General laboratory equipment.

### PROCEDURE

This procedure is far to manual or semi-automated coagulation systems. Follow the instructions of the instrument used.

For best results, duplicate samples are recommended.

1. Bring the sufficient volume of reagent to room temperature.
2. Prepare 1:10 dilution of the plasma (or plasma control) with Imidazol buffer (i.e. 25  $\mu$ L + 225  $\mu$ L).
3. Add **100  $\mu$ L** of the diluted plasma to the tube test.
4. Incubate the tube test at 37°C for 2 minutes.
5. Add **50  $\mu$ L** of the reagent and immediately starts the timer.
6. Record the clotting time.

For semi-automathic system refer to your instrument manual.

## CALCULATIONS

Calculate the mean clotting time of duplicate samples and controls. Differences between duplicate results should be less than 5%. Repeat the test if necessary.

Fibrinogen time can be reported in g/L, this dimension is calculated from a log-log calibration curve.

**Fibrinogen Calibration:** We recommend to use the MASTER CURVE enclosed in the kit.

Samples with concentrations <1 g/dL should be diluted 1:5 and assayed again.

## REFERENCE VALUES

Normal range: 2-4 g/L.

These values are for orientation purpose; each laboratory should establish its own reference range.

## QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures. They should be used as sample.

**REF** 3520101 PLASMA CONTROL LEVEL 1

**REF** 3520201 PLASMA CONTROL LEVEL 2

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

## CLINICAL SIGNIFICANCE

Fibrinogen, a protein synthesized by the liver, is the substance used in the blood to form a clot. Its determination is used to evaluate abnormal blood clotting.

Elevated Fibrinogen levels are observed in acute inflammations and in pregnancy; low values are observed in trombotic therapy, in hepatic disease, in the congenital non fibrinogen, in DIC (Disseminated Intravascular Coagulation) and in pancreatitis (low values).

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

## ANALYTICAL PERFORMANCE

- **Linearity** : 0.5 –5.7 g/L.

- **Analytical sensitivity**: 0.5 g/L.

- **Normal range**: 2.5 – 3.8 g/L.

- **Traceability** : Stago STA-Fibrinogen.

### - Accuracy

Results obtained with this reagent did not show significative differences when compared with reference reagents. Details of the comparison experiments are available on request.

## NOTES

1. If the clotting time of the 1:10 dilution of test plasma exceeds the clotting time of the last dilution point on the calibration curve, prepare a 1:5 dilution of test plasma and repeat the assay. Multiply the resulting value from the curve by 5 instead of 10 to allow for the different dilution factor. This will give the final concentration of the undiluted patient plasma.
2. If the clotting time of the 1:10 dilution of test plasma is shorter than the clotting time of the last dilution point on the calibration curve, prepare a 1:20 dilution of test plasma and repeat the assay. Multiply the resulting value from the curve by 20 instead of 10 to allow for the different dilution factor. If other dilutions are tested, the value obtained should be multiplied by the appropriate dilution factor.

## LIMITATIONS OF PROCEDURE

- The lowest recommended dilution is 1:3. Undiluted plasma cannot be tested because interfering substances and inhibitors may affect the accuracy of the results. Results are not significantly affected by the usual therapeutic levels of heparin up to 3.0 U/mL as found in anticoagulated patients. Prolonged clotting times will result at approximately 5 U/mL in the undiluted patient sample. Fibrin degradation products (FDP) may inhibit the thrombin action on fibrinogen and fibrin polymerization. In samples with normal fibrinogen levels, FDP has minimal effect; however, in samples with fibrinogen concentrations below 150 mg/dl and FDP concentrations greater than 100 µg/mL, the assay may be increasingly inhibited. Further dilution of the test plasma will reduce this interference.

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**Fibrinogen** **MASTER CURVE**

Introduzca los siguientes valores en el coagulómetro

Enter the following values in the coagulometer

LOT N°	35021
Expiry date	2010-09
g/L	SEC.
4	7.9
3	10.5
2	15.6
1	30.7