QuikCoag™ Fibrinogen

CE IVD For *In Vitro* Diagnostic Use

REF	Catalog Number	Quantity
	C.BMD.FIBR-02ML-8A	10 X 2 mL

INTENDED USE

QuikCoag Fibrinogen is an *in vitro* diagnostic assay intended for quantitative determination of fibrinogen in plasma.

SUMMARY

Thrombin converts soluble fibrinogen into insoluble fibrin, which when cross-linked becomes the fibrin clot as the last step in the coagulation cascade. Fibrinogen is an acute-phase reactant protein in that the concentration rises sharply in response to many different physiological stimuli such as tissue inflammation or injury. High fibrinogen levels are associated with atherosclerotic cardiovascular disease and with the occurrence of myocardial infarction and stroke. Other conditions in which fibrinogen is elevated are cancers of the stomach, breast, or kidney, and inflammatory disorders like rheumatoid arthritis. Reduced fibrinogen levels are prevalent in liver disease, prostate cancer, lung disease, bone marrow lesions, malnourishment, and disseminated intravascular coagulation. Other conditions of deficient fibrinog en are congenital afibrinogenemia, hypofibrinogenemia, and dysfibrinogenemia.

PRINCIPLE

QuikCoag Fibrinogen is based on the Clauss method of quantifying plasma fibrinogen. The Clauss method measures the rate of fibrinogen fibrin conversion in the presence of excess thrombin and has been shown to be rapid, sensitive and precise. When diluted plasma is clotted with excess thrombin, the fibrinogen level is inversely proportional to the clotting time. A calibration curve is prepared from a fibrinogen reference and plotted on log-log paper. This calibration curve is used to determine the fibrinogen concentration in the test sample.

REAGENTS

1. The fibrinogen reagent is a lyophilized preparation of bovine thrombin, approximately 100 NIH U/mL, buffer, stabilizers and preservative. Reconstitute individual vials with 2 mL of high purity water. Allow to stand at room temperature for 30 minutes before use. Ensure all particulate matter is well dissolved before use. After reconstitution, stable for 5 days at 2-8°C, or frozen for up to 30 days. Warm to room temperature prior to re-use.

PRECAUTIONS

Do not ingest. Avoid contact with skin, eyes or clothing. The fibrinogen control is a potentially biohazardous material. Source materials from which this product was manufactured were found negative for HBsAg and for antibodies against HCV, HIV-1 and HIV-2 using approved methods; however, no test method can offer complete assurance that infectious agents are absent. As with all materials of human origin, this product should be handled as a potentially infectious material.

SPECIMEN COLLECTION AND PREPARATION

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

1. Blood Collection using Syringe Method: 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.

2. Blood Collection using an Evacuated Blood Collection Tube: 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.

3. 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 cubic milliliter or red cells is not suitable for coagulation testing.

4. 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 immediately. The samples must not have any contact with glass.

PROCEDURE

Materials Required But Not Provided

1. Fibrinogen Control Normal
2. Imidazole Buffer

This procedure pertains to manual or semi-automated coagulation systems. Refer to your instrument manual for more detailed instrument specific instructions.

1. Prepare 1/5, 1/10, 1/20, 1/40 dilutions of fibrinogen control normal using the following table:
2.

	Normal Plasma	Imidazole Buffer	Total Volume
1/5	100 µL	400 µL	500 µL
1/10	100 µL	900 µL	1000 µL
1/20	100 µL	1900 µL	2000 µL
1/40	100 µL	3900 µL	4000 µL

3. Ensure the reconstituted fibrinogen is at room temperature prior to use.
4. Pipette 200 µL of each plasma dilution into a test cuvette.
5. Incubate at 37°C for 2 minutes. (Not longer than 5 minutes)
6. Rapidly add 100 µL of the room temperature fibrinogen, simultaneously starting the timer.
7. Record the clotting time in seconds. All samples should be done in duplicate.
8. Create a standard curve, plotting the average clotting time against fibrinogen concentration on a log-log graph. Plot the concentration (mg/dl) on x-axis and clotting time (sec) on the y-axis. Use the assigned fibrinogen value on the normal control to determine fibrinogen values for the dilutions. If the calibrated normal control has 286 mg/dl of fibrinogen undiluted, then multiply the 286 by the dilution factor to determine the fibrinogen content. Use the following table as an example, assuming the normal control was assigned a fibrinogen concentration of 286 mg/dl.

Dilution Factor	Conc. mg/dl
1/5	572
1/10	286
1/20	143
1/40	71.5

9. Prepare 1/10 dilutions for each patient plasma and test in duplicate as instructed in steps 3-7.
10. Using the average clotting time of the patient plasma, extrapolate the fibrinogen value from the standard curve. Multiply the value from the curve by 10 to determine the undiluted patient fibrinogen value.

QUALITY CONTROL

Reliability of the calibration curve should be monitored within each run using normal and abnormal fibrinogen control plasmas. Each

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laboratory should establish a control range to determine the allowable variation in day-to-day performance of each control plasma. The standard curve should be prepared on a monthly basis or when a new lot of reagent is used to assure proper performance.

LIMITATIONS

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, make 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.

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, make 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.

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.

EXPECTED VALUES

The normal range for fibrinogen levels in human plasma is considered to be 200-400 mg/dl. Each laboratory should establish its own mean normal and normal range because of variances among different laboratories.

PERFORMANCE CHARACTERISTICS

Precision: Within-run precision was assessed using normal fibrinogen and abnormal fibrinogen controls on both optical and a mechanical instruments. The results are shown in the following table:

Within-run Precision Results

Sample	Coatron IV (Optical)	Amelung (Mechanical)
Fibrinogen Control Normal	3.9 %	2.5 %
Fibrinogen Control Low Abnormal	2.7 %	1.8 %

Correlation: Correlation studies were performed against the Fibrinogen reagent of a competitor on the Coatron IV coagulometer. The results are shown in the following table.

Correlation Results

Regression coefficient	Slope	Intercept
0.920	0.99	0.06

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Symbols Key	
	Manufactured By
	Consult Instructions For Use
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
	Expiration Date (YYYY.MM)
	Temperature Limitations
REF	Catalogue Number
CON	Contents
REC	Reconstitution Volume
	Biological Risks
EC REP	European Authorized Representative