FIBRINOGEN KIT

DIAGNOSTIC KIT FOR DETERMINATION OF FIBRINOGEN CONCENTRATION

Kit name	Kit size	Cat. No
FIBRINOGEN KIT-10	100 assays	K-500
FIBRINOGEN KIT-30	300 assays	K-550

INTRODUCTION

The enzyme, thrombin, is the penultimate protein in the clotting sequence, acting upon soluble fibrinogen and converting it to insoluble fibrin. Normal plasma fibrinogen levels range from 200-400 mg/dl, although levels as low as 10-20 mg/dl may occur in acquired or congenital hypofibrinogenemia. The determination of plasma fibrinogen levels has proven to be a useful test in the diagnosis of hyperfibrinogenemia, hypofibrinogenemia, dysfibrinogenemia, afibrinogenemia.

METHOD PRINCIPLE

The thrombin clotting time fibrinogen assay is based on the method originally described by Clauss. In the presence of high concentrations of thrombin, the time required for clot formation in dilute plasma is inversely proportional to the fibrinogen concentration.

REAGENTS

Package

	FIBRINOGEN KIT-10
Bovine thrombin 100	5 x 2 ml
Imidazole buffer	1 x 135 ml
Low fibrinogen control	3 x 1 ml
High fibrinogen control	1 x 1 ml

	FIBRINOGEN KIT-30
Bovine thrombin 100	6 x 5 ml
Imidazole buffer	2 x 135 ml
Low fibrinogen control	3 x 1 ml
High fibrinogen control	1 x 1 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package.

Reagents preparation and stability

a) Bovine thrombin 100 (100 NIH U/ml) – 2 ml or 5 ml:

Lyophilized buffered bovine thrombin.

Reconstitute with distilled water according to vial label. Agitate gently until solution is complete. The reconstituted material is stable for 7 days at 2-8°C or 8 hours at 15-30°C or 30 days at -20°C. Thaw rapidly at 37°C. Do not refreeze.

b) Imidazole buffer:

Imidazole buffer is ready to use; pH 7.4, with 0.1% sodium azide as a preservative.

c) Low fibrinogen control:

Processed from human plasma collected with sodium citrate anticoagulant (4% w/v). Reconstitute with 1.0 ml of high purity water. Swirl gently and let stand undisturbed for 15 minutes at room temperature. Do not invert vial or mix vigorously. After proper reconstitution, Low fibrinogen control is stable for 16 hours when stored in capped vial at 2-8°C. Undiluted plasma can also be stored frozen. Thaw quickly at 37°C and swirl gently to mix. Do not refreeze.

d) High fibrinogen control:

Processed from human plasma collected with sodium citrate anticoagulant (4% w/v). Reconstitute with 1.0 ml of high purity water. Swirl gently and let stand undisturbed for 15 minutes at room temperature. Do not invert vial or mix vigorously. After proper reconstitution, High fibrinogen control is stable for 8 hours when stored in capped vial at 2-8°C. A precipitate may form when refrigerated. Gently warm the plasma to 37°C to minimize any precipitate. Undiluted plasma can also be stored frozen. Thaw quickly at 37°C and swirl gently to mix. Do not refreeze.

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- **Low fibrinogen control** is formulated to have a fibrinogen value of approximately 70-120 mg/dl. **High fibrinogen control** should be approximately 500 to 700 mg/dl. Actual values recovered depend on the instrument and reagent used.
- Products from human source have been tested for the HIV antibody, HbsAg and HCV and found to be non-reactive. However this material should be handled as thought capable of transmitting infectious disease.
- Imidazole buffer contains sodium azide (< 0.1 %) as a preservative. Avoid contact with skin and mucous membranes.
- The reagents are designed to work at 37°C. Frequently check the temperature of all heating elements.
- Reagents BOVINE THROMBIN 100 (Cat. No K-503) and IMIDAZOLE BUFFERED SALINE (Cat. No K-504) can be ordered separately.
- Imidazole buffer meeting the criteria for classification in accordance with Regulation (EC) No 1272/2008.

Ingredients: Imidazole

Danger

H360FD May damage fertility. May damage the unborn child.

P201: Obtain special instructions before use.

P202: Do not handle until all safety precautions have been read and understood.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P308+P313: IF exposed or concerned: Get medical advice/attention.

P405: Store locked up.

P501: Dispose of the contents/containers in accordance with the current legislation on waste treatment.

ADDITIONAL EQUIPMENT

- CALIBRATION PLASMA-NORMAL LEVEL (Cat. No K-720);
- a mechanical or photo-optical means of clot detection;
- general laboratory equipment.

SPECIMEN

A. Anticoagulant- sodium citrate - 3.2% (0.105M).

B. Specimen collection:

1. Obtain venous blood.
2. Immediately mix 9 parts blood with 1 part anticoagulant, mix well by inversion of tube.
3. Centrifuge the specimen at 1000 rcf for 15 min.
4. Remove plasma from the tube within 60 min using a plastic pipette and store in a plastic tube.
5. Test plasma sample within 2 hours, otherwise store frozen and thaw just prior to use.

PROCEDURE

Kit is suitable for use with manual, mechanical or automated instrument for clot detection. See instrument manufacturers instructions for full details.

Manual and mechanical assay

1. Prepare a minimum of five different dilutions of the reconstituted CALIBRATION PLASMA NORMAL (Cat. No K-720) in imidazole buffer.
2. Dilute quality control and patient samples 1:10 in imidazole buffer.
3. Prewarm 0.2 ml of each dilution to 37°C for 4-6 minutes.

4. Add 0.1 ml of bovine thrombin reagent 100 to prewarmed dilution and time clot formation. Do not prewarm thrombin.
5. Report clotting times for each dilution to the nearest 0.1 second.
6. Prepare a calibration curve from results of calibration plasma. The frequency of curve preparation is partially determined by the method of clot detection used. Always prepare a new curve with each change in reagent lots, instrumentation, or when controls no longer fall within established ranges.

Calculation

A) Plasma diluted 1:10 represents 100% of the assigned value. The dilution factor indicates the relationship between the 1:10 dilution and other dilutions, eg:
standard = 304 mg/dl fibrinogen (each lab must prepare curves with their reagents and instrumentation).

Dilution	Dilution factor	Fibrinogen (mg/dl)	Mean clotting time (s)
1:3.5	10/3.5 = 2.6	304 x 2.6 = 790	5.8
1:5	10/5 = 2	304 x 2 = 608	7.3
1:10	10/10 = 1	304 x 1 = 304	13.4
1:15	10/15 = 0.67	304 x 0.67 = 204	20.8
1:35	10/35 = 0.29	304 x 0.29 = 88	49.2

B) Use all five of the calibrator points to construct a log-log curve that plots fibrinogen concentration vs. clotting time. Draw the straight line of best fit. Examine the curve and, if necessary, omit non-linear points. The final curve must consist of at least three consecutive points.

C) Find the clotting time of quality control and patient samples on the curve and read the corresponding fibrinogen value. If clotting times for the 1:10 dilution fall outside the linear curve, prepare 1:5 or 1:20 dilutions as needed. If the sample is diluted 1:5, divide the result from the standard curve by 2; if the sample was diluted 1:20, multiply the curve result by 2 to get the final result.

REFERENCE VALUES[7]

Generally, the normal reference interval is 150 to 350 mg/dl (1.5 to 3.5 g/l).
Laboratories should establish a normal reference interval for fibrinogen measurements.

QUALITY CONTROL

For calibration the CALIBRATION PLASMA NORMAL (Cat. No K-720) it is recommended.
For internal quality control it is recommended to use the normal and abnormal control plasma, such as: CONTROL PLASMA NORMAL (Cat. No K-100), low fibrinogen control, high fibrinogen control with each batch of samples.

LIMITATIONS

A. Blood must be immediately added to trisodium citrate anticoagulant and gently mixed. EDTA and heparin are unsuitable anticoagulants.
B. Hemolysis can cause clotting factor activation and end point detection interference.
C. Icteric and lipemic specimens may also be inappropriate for end point detection methods.
D. The ratio of blood to anticoagulant is usually 9:1 and results in a citrate concentration of 10.9 to 12.9 mmol/l. This concentration must be adjusted for patients with hematocrits above 55%.
E. Freezing and thawing of plasma that contains residual cells will generate damaged cell membranes that can affect results.
F. Acute inflammatory reactions can elevate circulating Factor I (fibrinogen).
G. High Fibrinogen Degradation Products (FDP) may prolong clotting times, especially when the fibrinogen level is below 150 mg/dl.
H. In patients with qualitative fibrinogen abnormalities, the thrombin clotting time assay may indicate decreased fibrinogen. The quantitative fibrinogen results may be normal on these same samples if tested by other methods.
I. Heparin does not interfere at therapeutic levels. However, very high heparin levels may cause low fibrinogen results. Batroxobin enzyme can be substituted for thrombin in this assay if heparin interference is suspected.
J. High paraprotein levels, thrombin antibodies, and drugs that activate the fibrinolytic system can interfere with fibrinogen assays.

PERFORMANCE CHARACTERISTICS

1. Accuracy:

A low, a normal, and a high fibrinogen plasma were tested in multiple laboratories using CORAMY reagents. The results were compared to results obtained using other manufacturer's reagents in multiple labs.

Level	CORMAY reagents	n	Other reagents	n
Low	144 mg/dl	10	163 mg/dl	195
Normal	294 mg/dl	10	297 mg/dl	195
High	488 mg/dl	16	474 mg/dl	390

2. Precision:

A low, a normal, and a high fibrinogen plasma were tested on multiple days using CORMAY reagents on a photo-optical instrument. Ten standard curves were determined on each test day,for a total of 30 standard curves.
CV = 5.9% (Low level)
CV = 3.4% (Normal level)
CV = 2.9% (High level)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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