



Intended Use

Diagen Fibrinogen Determination Kit is designed for the quantitative determination of Fibrinogen concentration.

Summary and Principle

Fibrinogen, an essential plasma protein, is synthesised in the hepatocyte and comprises of three different polypeptide chains (A α , B β and γ); linked by disulphide bridges, of which there are two. Thrombin cleaves the A α and B β chains releasing fibrinopeptides A and B, respectively, from the amino-terminal ends. Once the fibrinopeptides are released, the resulting fibrin monomers undergo polymerization and form an insoluble fibrin clot⁽¹⁾.

Diagen Fibrinogen Determination Kit uses an excess of thrombin to convert fibrinogen to fibrin in diluted plasma. The clotting time of a dilution of plasma with a standard concentration of thrombin is inversely related to the fibrinogen concentration. At high thrombin and low fibrinogen concentration, the rate of reaction is a function of fibrinogen concentration. This method was first outlined by Claus in 1957⁽²⁾.

Reagent

Standard Fibrinogen Plasma 2 vials
A buffered, lyophilised human plasma with a standard fibrinogen concentration. For reconstitution add 0.5 mL of distilled water, replace stopper and swirl gently. Allow 5 to 10 minutes for complete solution.

Lot Number: FS01
Fibrinogen Concentration: 2.75g/L

Bovine Thrombin Reagent 6 vials

A lyophilised preparation of bovine thrombin (20 units/ml) containing polybrene. For reconstitution, add 1.0 mL of distilled water replace the stopper and swirl gently. Allow 5 to 10 minutes for complete solution.

Concentrated Imidazole Buffer 2 vials
Concentrated Imidazole buffer, with a pH of 7.35. **In preparation for use dilute the solution 1 in 5 with distilled water.**

Warnings and Precautions

POTENTIAL BIOHAZARD MATERIAL.

Diagen Fibrinogen Standard Plasma is of human origin. All donor units used in production of this product have been found negative for anti HIV, anti HCV, HBsAg and Syphilis by approved methods. However, all plasma of human origin should be considered as potentially infectious and handled appropriately. Please refer to the relevant SDS Sheet for handling and safety procedures. Dispose of all waste materials according to the stated international, national and local regulations.

Diagen Thrombin Reagent is of bovine origin. Reagents containing animal by-products should be treated as potentially infectious. All wastes containing biological material should be correctly labeled and stored separately from other wastes. Waste materials should be disposed of according to prescribed international, national and local regulations. Please refer to the SDS Sheet (provided on request) for handling and safety procedures.

Collection of Blood Samples

Blood (9 parts) is collected into 1 part of 3.2% trisodium citrate and the plasma obtained by centrifugation at 2500 g for 15 minutes.

The plasma should be stored in stoppered tubes. The use of 3.2% citrate containing 5% HEPES buffer improves the stability of both fresh and deep frozen plasma.

Procedure

Materials Provided

Materials needed for Claus Fibrinogen test are detailed below:

Cat. No.
FIB440 Fibrinogen Estimation Kit

Materials and equipment required, but not provided:

1. General routine laboratory coagulation equipment.
2. Reaction cups or test tubes (12 x 75 mm).
3. Pipette delivering between 100 μ L, 200 μ L and 1.0 mL.
4. Distilled water.
5. Diagen Reference Control Plasma - Normal (RCPN070).
- Abnormal (RCPA080).

Manual Technique

1. Make 1 in 10 dilutions of the test and standard fibrinogen plasma in Imidazole buffer.
2. Warm 200 μ L of diluted plasma at 37°C for 2 minutes.
3. Add 100 μ L of thrombin reagent to the plasma dilutions and measure the clotting time in the normal way.

Please note:

1. Tubes should be new and scrupulously clean.
2. Water bath temperature should be 37°C.
3. Diagen Normal and Abnormal Reference Control plasmas can be used as part of a quality control system for this assay.

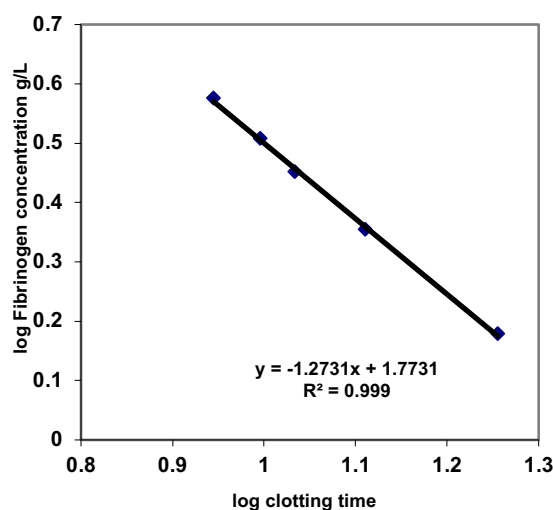
Interpretation of results

The fibrinogen concentration is then read off of the calibration chart provided – see Figure 2. **The calibration chart provided is for manual use only** – for coagulometers, a calibration curve must be produced in-house by making appropriate dilutions of the standard plasma supplied and plotting the log of fibrinogen concentration against the log clotting time, see example below.

Example: Standard Plasma = 2.26 g/L

Plasma DILUTION	Secs. (X axis)	g/L (Y axis)	log X	log Y
1/6.	8.8	3.76	0.9444827	0.5751878
1/7.	9.9	3.22	0.9956352	0.5078559
1/8.	10.8	2.83	1.0334238	0.4517864
1/10.	12.9	2.26	1.1105897	0.3541084
1/15.	18	1.51	1.2552725	0.1789769

log Fibrinogen concentration v log Clotting time



The standard plasma (lot & fibrinogen concentration on Page 1) is used as a control.

For clotting times of less than 7.5 seconds, make a 1 in 15 dilution of plasma and multiply the fibrinogen concentration from the calibration chart by 1.5.

For clotting times of more than 17 seconds, make a 1 in 7.5 dilution of plasma and multiply the fibrinogen concentration from the calibration chart by 0.75.

The normal range for Fibrinogen using the Clauss technique is: 1.7 – 4.0 g/L

Figure 2. The Manual Calibration Chart for Thrombin reagent.

Lot: F
Expiry: **/****

Seconds	Fib g/L
7.5	3.89
7.6	3.82
7.7	3.75
7.8	3.68
7.9	3.61
8.0	3.54
8.1	3.48
8.2	3.42
8.3	3.36
8.4	3.30
8.5	3.24
8.6	3.19
8.7	3.14
8.8	3.08
8.9	3.03
9.0	2.98
9.1	2.94
9.2	2.89
9.3	2.85
9.4	2.80
9.5	2.76
9.6	2.72
9.7	2.68
9.8	2.64
9.9	2.60
10.0	2.56
10.1	2.52
10.2	2.49
10.3	2.45
10.4	2.42
10.5	2.38
10.6	2.35
10.7	2.32
10.8	2.29
10.9	2.26
11.0	2.23
11.1	2.20
11.2	2.17
11.3	2.14
11.4	2.11
11.5	2.09
11.6	2.06
11.7	2.04
11.8	2.01
11.9	1.99
12.0	1.96
12.1	1.94
12.2	1.92
12.3	1.89
12.4	1.87
12.5	1.85
13.0	1.75
13.5	1.65
14.0	1.57
14.5	1.49
15.0	1.42
15.5	1.35
16.0	1.29
16.5	1.23
17.0	1.18

Decrease in Fibrinogen concentration is found in:

- DIC - consumption of clotting factors.
- Liver disease - decreased synthesis.
- Massive transfusion - dilutional coagulopathy.
- Inherited deficiency - eg hypofibrinogenaemia, afibrinogenaemia & dysfibrinogenaemia.
- Following thrombotic therapy.

Increase in Fibrinogen concentration is found in: -

- Increasing age.
- Pregnancy or oral contraception.
- In post-menopausal women.
- Acute phase reaction.
- Disseminated malignancy (but may be decreased if associated with DIC).

Quality Control

All laboratories should have in place a quality control system that uses normal and abnormal controls to evaluate reagent, instrument and user performance. Both normal and abnormal controls should be used prior to performing a test series to validate the patient results. We recommend Diagen normal and abnormal control plasmas for this purpose, as these have been specifically manufactured for our test kits. If the controls do not perform within their reference ranges, a review of the instrument or test system is recommended and analysis repeated.

Limitations

Diagen Fibrinogen Determination test is unaffected by heparin up to concentrations of 2 u/mL in plasma due to an added heparin inhibitor. However, heparin levels greater than this, Fibrinogen Degradation Products (FDPs) and other inhibitors may give falsely low concentrations.

Storage and stability

The unopened freeze dried vials are best stored deep frozen, but may be stored for up to 3 years at 2 - 8°C without deterioration. Once reconstituted, the contents of the vial are stable for up to 24 hours when held at 2 - 8°C. The product may be deep frozen at -20°C and thawed once.

Packaging









Each kit contains:

- 2 x 0.5 mL vials of Fibrinogen Standard Plasma.
- 6 x 1.0 mL vials of Bovine Thrombin Reagent.
- 2 x 5.0 mL vial of Concentrated Imidazole Buffer – pH 7.35.

References

1. Hantgan, R.R., Francis, C.W. & Marder, V.J. (1994) Fibrinogen structure and physiology. In: Hemostasis and Thrombosis: Basic Principles & Clinical Practice (ed. by R.W. Colman, J. Hirsh, V.J. Marder & E.W. Salzman), pp. 277–300. JB Lippincott, Philadelphia.
2. Clauss A. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens, Acta Haemat. 1957;17: 237.

Key guide to symbols

 REF	Manufacturers catalogue number.		Consult instructions for use.
 LOT	Manufacturers batch number.		Requires reconstitution.
 IVD	For <i>in vitro</i> diagnostic use only.		Product expiry date.
	Biological risks.		Keep refrigerated between 2-8°C.

 Manufacturer.

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