



**Fibrinogen Assay Set (100 or 300 determinations)
or one of the following components:
Fibrinogen Reference Plasma
Bovine Thrombin (200 or 500 NIH per vial)
Imidazole Buffered Saline**

I. Intended Use

Pacific Hemostasis® Fibrinogen Assay Set, Fibrinogen Reference Plasma, Imidazole Buffered Saline (IBS), and Bovine Thrombin 200 and 500 are intended for use in the quantitative determination of fibrinogen in plasma samples.

II. Summary and Principles

The thrombin clotting time fibrinogen assay is based on the method originally described by Clauss.¹ In the presence of high concentrations of thrombin, the time required for clot formation in dilute plasma is inversely proportional to the fibrinogen concentration.

III. Reagents

For *in vitro* diagnostic use.

Store all unopened reagents at 2-8°C.

Composition:

Bovine Thrombin 200 or 500 (Approximately 100 NIH Units/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 may be frozen within 4 hours for use within 30 days.⁵ Thaw rapidly at 37°C. Do not refreeze.⁶

Fibrinogen Reference Plasma: Human plasma collected with sodium citrate anticoagulant. <1.0% Stabilizers and buffers are added prior to lyophilization. Reconstitute with 1.0 mL distilled water. Agitate gently until solution is complete. Reconstituted material is stable for 8 hours at 2-8°C.⁷

Imidazole Buffered Saline (IBS): Imidazole buffer in saline, pH 7.4 ± 0.2, with 0.1% sodium azide as a preservative.

Caution: Each unit of source material used in the preparation of Fibrinogen Reference Plasma has been tested by an FDA licensed method and found non-reactive for HBsAg and negative for antibodies to HIV and HCV. However, no known test method can offer complete assurance that products derived from human blood will not transmit hepatitis, AIDS, or other infectious diseases. This product, like all materials of human origin, should be handled as potentially infectious biological material.

Warning: IBS contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Dilute with running water before discarding, flush with large volumes of water. These precautions are recommended to avoid deposits in metal piping in which explosive conditions may develop.

Lack of vacuum in the Fibrinogen Reference Plasma vials or Thrombin, erratic values, or product color variations could indicate deterioration. However, poor performance could also be due to other factors within the test system.

IV. Specimen Collection

3.2% (0.109M) trisodium citrate anticoagulant is recommended for coagulation assays. Avoid hemolysis and contamination by tissue fluids. Samples that have less than 90% of the expected

fill volume should be rejected. Centrifuge blood for 15 minutes at 1500 x g. Test within 2 hours if samples are held at 22-24°C. For more details on specimen collection and storage, see NCCLS Document H21-A3.²

V. Test Procedure

Materials Provided:

Pacific Hemostasis Fibrinogen Assay Set : 100 determinations:

Bovine Thrombin 200: five 2.0 mL vials
Fibrinogen Reference Plasma: three 1.0 mL vials
Imidazole Buffered Saline: one 135 mL vial

Pacific Hemostasis Fibrinogen Assay Set : 300 determinations:

Bovine Thrombin 500: six 5.0 mL vials
Fibrinogen Reference Plasma: two 1.0 mL vials
Imidazole Buffered Saline: two 135 mL vials

Note: Bovine Thrombin 200, Bovine Thrombin 500, Fibrinogen Reference Plasma, and Imidazole Buffered Saline can also be purchased separately.

Materials Required, But Not Provided:

Plastic test tubes
Precision pipeters: 0.1 mL and 0.2 mL
Serological pipets
Normal and abnormal controls, such as Pacific Hemostasis Level 1 Coagulation Control, Low Fibrinogen Control, and High Fibrinogen Control Plasmas.

The Fibrinogen Assay Set and individual components are suitable for use with manual, mechanical or photo-optical methods of end-point clot detection. For automated and semi-automated instruments, follow the instrument manufacturer's instructions. For manual and mechanical test methods:

- Prepare a minimum of five different dilutions of the reconstituted Fibrinogen Reference Plasma in IBS. Dilute plasma at least 1:3 to minimize interfering factors.³
- Dilute quality control and patient samples 1:10 in IBS.
- Prewarm 0.2 mL of each dilution to 37°C for 4-6 minutes.
- Add 0.1 mL of Bovine Thrombin reagent to prewarmed dilution and time clot formation. Do not prewarm thrombin.
- 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.³

VI. Results

- Plasma diluted 1:10 represents 100% of the assigned value. The dilution factor indicates the relationship between the 1:10 dilution and other dilutions.

Example Only:

Standard = 304 mg/dL fibrinogen (Each lab must prepare curves with their reagents and instrumentation.)⁸

Dilution	Dilution factor	Fibrinogen (mg/dL)	mean CT (seconds)
1:3.5	10/3.5 = 2.9	304 x 2.9 = 882	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

- Calculate the mean of duplicate clotting times to the nearest 0.1 second. 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. Constructing the curve with only the most linear points will produce the best recovery on control and patient samples.

- 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.

VII. 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. Icteric and lipemic specimens may also be inappropriate for end point detection methods.
- C. The sample should only contact nonwetable surfaces.
- 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%. See NCCLS Document H21-A3.^{1,2}
- 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).³
- G. High Fibrinogen Degradation Products (FDP) may prolong clotting times, especially when the fibrinogen level is below 150 mg/dL.³
- 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.^{3,4}
- I. Heparin does not interfere at therapeutic levels. However, very high heparin levels may cause low fibrinogen results.³ 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.³

- K. The Fibrinogen Assay Set and individual components are designed to work at 37° C. Ensure that all heating elements are functioning properly.

VIII.Expected Values: Laboratories should establish a normal reference interval for fibrinogen measurements. Generally, the normal reference interval is 150 to 350 mg/dL (1.5 to 3.5 g/L).³

IX. Performance Characteristics

- A. Accuracy:** A low, a normal, and a high fibrinogen plasma were tested in multiple laboratories using Pacific Hemostasis reagents. The results were compared to results obtained using other manufacturer's reagents in multiple labs.⁹

Sample	Pacific Hemostasis	n =	All 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

- B. Precision:** A low, a normal, and a high fibrinogen plasma were tested on multiple days using Pacific Hemostasis reagents on a photo-optical instrument. Ten standard curves were determined on each test day, for a total of 30 standard curves. The percent CV was determined to be 5.9% (low), 3.4% (normal), and 2.9% (high).¹⁰

X. References

1. Clauss, A. *Acta Haemat.* 17:237-246, 1957.
2. NCCLS: *Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays.* 2nd edition. Approved guideline. NCCLS Document H21-A3. Wayne, PA., 1998.
3. NCCLS: *Procedure for determining fibrinogen in plasma.* Approved guideline. NCCLS Document H30-A2. Wayne, PA., 2001.
4. Musgrave, K.A., Bick, R.L.: *Quality Assurance in the Hemostasis Laboratory.* In Bick, R.L., et al, editors: *Hematology: Clinical and Laboratory Practice.* Vol. 2, pp1309-1315. Mosby. St. Louis, MO., 1993.
- 5-7. Stability data found in DHF.
- 8-10. Data found in 510K file

ORDERING INFORMATION

Cat. No.	Description	Contents
100600	Fibrinogen Assay Set	100 determinations
100605	Fibrinogen Assay Set	300 determinations
100602	Fibrinogen Reference Plasma	10 x 1 mL
100604	Low Fibrinogen Control Plasma	10 x 1 mL
100607	High Fibrinogen Control Plasma	10 x 1 mL
100595	Coagulation Control Plasma, Level 1	10 x 1 mL
100601	Bovine Thrombin 200 (100 NIH/mL)	10 x 2 mL
100606	Bovine Thrombin 500 (100 NIH/mL)	10 x 5 mL
100647	Imidazole Buffered Saline	2 x 135 mL

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