Enzymatic Assay of ANCROD
(EC 3.4.21.28)

PRINCIPLE:

Fibrinogen $\xrightarrow{\text{Ancrod}}$ Fibrin

CONDITIONS: T = 37°C, pH 7.35

METHOD: Fibrometer

REAGENTS:

A. 143 mM 5,5-Diethylbarbiturate and 143 mM Sodium Acetate Solution (Soln A)
(Prepare 275 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, and Barbital, Sodium Salt, Sigma Prod. No. B-0500.)

B. 4.25% (w/v) Sodium Chloride Solution
(Prepare 300 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)

C. 36 mM 5,5-Diethylbarbiturate, 36 mM Sodium Acetate, 0.85% (w/v) Sodium Chloride Solution, pH 7.35 at 25°C (Soln B)
(Prepare 1 liter by combining 250 ml of Reagent A, 200 ml of Reagent B, 217 ml of 0.1 M HCl, and 333 ml of deionized water. Adjust the pH to 7.35 at 37°C with either 1 M HCl or 1 M NaOH.)

D. 25.7 mM Sodium Citrate Solution
(Prepare 100 ml in deionized water using Citric Acid Trisodium Salt, Dihydrate Sigma Prod. No. C-7254.)

E. 0.85% (w/v) Sodium Chloride Solution (NaCl)
(Prepare 300 ml in deionized water using Reagent B.)

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REAGENTS:

F. 7.2 mM 5,5-Diethylbarbiturate, 7.2 mM Sodium Acetate, 5.1 mM Sodium Citrate, 0.68% (w/v) Sodium Chloride, 1.0% (w/v) Bovine Serum Albumin, and 0.5% (w/v) Polyethylene Glycol (Enz Dil)
(Prepare 500 ml by combining 100 ml of Reagent C, 100 ml of Reagent D, 300 ml of Reagent E, Albumin Bovine, Sigma Prod. No. A-4378, and Polyethylene Glycol Sigma Prod.
No. P-2139.)

G. Plasma Solution (Plasma)
(Immediately before use, reconstitute 2.0 ml of Human Plasma, lyophilized with 2 ml of deionized water. Then add 2 ml of Reagent E. Keep at room temperature.)

H. Ancrod Enzyme Solution (Ancrod)
(Immediately before use, reconstitute the Ancrod vial with 1 ml of deionized water. Further dilute to 2.7 - 5.1 units/ml of Ancrod in Reagent F, in glass tubes. Serial dilutions should be made in Reagent F so that clotting times are between 15 - 25 seconds. If the clotting time recorded is too long, increase the Ancrod concentration. If the time recorded is too short, decrease the Ancrod concentration. Subsequent dilutions are also to be made in glass tubes.)

I. NIH Thrombin Standard Solution (Std)
(Use Thrombin Reference Standard (Lot J) which has been diluted in Reagent F. Note: The current Standard Curve has been made with N.I.H. Thrombin Standard diluted in glass test tubes. Next Standard Curve (late 1995) will use Plastic test tubes.[1])

PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagents into fibrometer cups, which have been pre-warmed to 37°C.

	Test
Reagent G (Plasma)	0.20

Allow to stand at 37°C for thirty seconds. Then add:

Reagent H (Ancrod)	0.10

PROCEDURE: (continued)

Record the clotting time using a suitably thermostatted fibrometer. A 0.3 ml fibrometer probe should be used.

Compare the clotting times of the Tests against a NIH Standard Curve for Thrombin clotting times versus units of Thrombin/ml.

Determine the units of Ancrod of the Test from the Standard Curve.

CALCULATIONS:

Units/ml enzyme = (Units of Ancrod from Std Curve)(df)

df = Dilution factor

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

Activity is expressed in NIH units obtained by direct comparison to a NIH Thrombin Reference Standard, Lot J.

FINAL ASSAY CONCENTRATIONS:

In a 0.30 ml reaction mix, the final concentrations are 33% (v/v) plasma, and 0.27 - 0.51 unit ancrod (components of the enzyme diluent are not included).

REFERENCE:

Human Blood Coagulation, Haemostasis, and Thrombosis (1976)
p. 721-722, 2nd ed., R. Biggs, ed., Blackwell Scientific Publications

NOTES:

1. Standards should be used immediately and kept on ice after they are dissolved.

NOTES: (continued)

2. This assay is based on the cited reference.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.