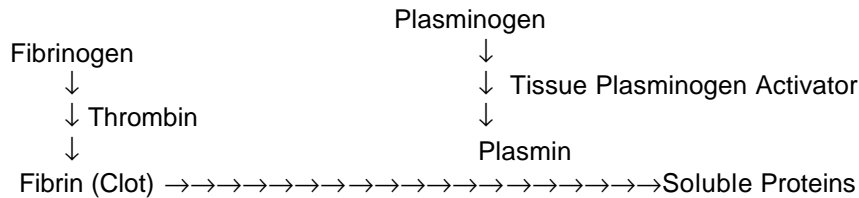


Enzymatic Assay of Tissue Plasminogen Activator Fibrinolytic Assay

PRINCIPLE:



CONDITIONS: T = 37°C, pH = 7.3

METHOD: Visual Fibrinolytic Endpoint Determination

REAGENTS:

- A. 0.1 M Sodium Phosphate/0.01% Tween 80 (BUF)
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Sigma Prod. No. S-0751 and Polyoxyethylenesorbitan, Monooleate (Tween 80), Sigma Prod. No. P-1754. Adjust to pH 7.3 at 37°C with 2 NaOH.)
- B. 9% (w/v) Fibrinogen Solution (FIB)
(Prepare 10 ml in Reagent A using Fibrinogen, Type I-S from Bovine Plasma, Sigma Prod. No. F8630. Allow solution to stir for at least one hour at 37°C.)
- C. 0.15 M Sodium Chloride Solution (NaCl)
(Prepare 50 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- D. 0.5 M Sodium Chloride Solution
(Prepare 50 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- E. 1.0 M Potassium Bicarbonate Solution
(Prepare 50 ml in deionized water using Potassium Bicarbonate, Sigma Prod. No. P-9144.)
- F. Thrombin Solution (THR)
(Prepare 4 ml at 250 units/ml in cold Reagent C using Thrombin from Human Plasma, Sigma Prod. No. T-6884. Keep on ice.)
- G. Plasminogen Solution (PLS)
(Prepare by dissolving a five unit vial in 1.0 ml of cold Reagent D using Plasminogen from Human Plasma, Sigma Prod. No. P-5661. After dissolution, dilute to 0.05 units/ml with cold Reagent A. Keep on ice.)

Enzymatic Assay of Tissue Plasminogen Activator Fibrinolytic Assay

REAGENTS: (continued)

- H. Tissue Plasminogen Activator Standard (t-PA Std)
(Prepare by dissolving a 10 µg vial, approximately 5000 units of t-PA per vial, in 1.0 ml of cold Reagent E using Chromogenix t-PA standard. Place on ice for 15 minutes and then dilute to approximately 2500 units/ml in cold Reagent A0)

- I. Tissue Plasminogen Activator Test Solutions
(Prepare solution containing approximately 500 units/ml in cold Reagent E. Place on ice for 15 minutes.¹ Immediately before use, prepare a solution containing approximately 2500 units/ml with cold Reagent A.)

PROCEDURE:

Clot Formation Check

Pipette (in milliliters) the following reagents into a suitable container:

	<u>Test</u>
Reagent B (FIB)	0.200
Reagent G (PLS)	0.125

Equilibrate at 37°C for 3 minutes. Then add:

Reagent A (BUF)	0.125
-----------------	-------

Equilibrate at 37°C for 1 minute. Then add:

Reagent F (Thrombin)	0.050
----------------------	-------

Mix by swirling and incubate at 37°C for approximately 2-3 minutes to allow for clot formation. Then gently add a glass bead to the top of the test reaction mixture. If the glass bead remains on top of the reaction test mixture, then the system is working properly. Proceed to the Clot Lysing Check. If the bead falls through the clot, increase the concentration of Fibrinogen in Reagent B and repeat Clot Formation Check.

Clot Lysing Check

Pipette (in milliliters) the following reagents into a suitable containers:

Reagent B (FIB)	0.200
Reagent G (PLS)	0.125

Enzymatic Assay of Tissue Plasminogen Activator Fibrinolytic Assay

PROCEDURE: (continued)

Equilibrate at 37°C for 3 minutes. Then add:

	Test
Reagent H (t-PA Std)	0.125

Equilibrate at 37°C for 1 minutes. Then add:

Reagent F (Thrombin)	0.050
----------------------	-------

Record initial time (T_i) in minutes, and mix by swirling and incubate at 37°C for approximately 2-3 minutes to allow for clot lysis. Then gently add a glass bead to the top of the test reaction mixture. Record time as final time (T_f) when the glass bead touches the bottom of the tube. If the change in time is between 5 to 10 minutes, then proceed with standard curve. The change in time is the difference between the recorded time upon addition of thrombin (T_i :Initial Time) and the time it takes for the glass bead to touch the bottom of the container (T_f :Final Time). If the change in time is not between 5 to 10 minutes, then prepare an appropriate dilution of the standard and repeat Clot Lysing Check.

Standard Curve

Pipette (in milliliters) the following reagents into a suitable container:

	Std1	Std2	Std3	Std4	Std5	Std6	Std7
Reagent B (FIB)	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Reagent G (PLS)	0.125	0.125	0.125	0.125	0.125	0.125	0.125

Mix by swirling and equilibrate at 37°C for 3 minutes. Then add:

Reagent A (BUF)	----	0.025	0.045	0.065	0.085	0.105	0.115
Reagent H (TPA)	0.125	0.100	0.080	0.060	0.040	0.020	0.010

Mix by swirling and equilibrate at 37°C for 1 minute. Then add:

Reagent F (THR)	0.050	0.050	0.050	0.050	0.050	0.050	0.050
-----------------	-------	-------	-------	-------	-------	-------	-------

Mix by swirling and incubate at 37°C for 2-3 minutes and record the initial time (T_i) in minutes and record the initial time (T_i) in minutes after addition of Reagent F. Gently add the glass bead to each container starting with Std 1. Record time as the final time (T_f) when the glass bead touches the bottom of the container.

Enzymatic Assay of Tissue Plasminogen Activator Fibrinolytic Assay

PROCEDURE: (continued)

Test

Pipette (in milliliters) the following in a suitable container:

	<u>Test 1</u>	<u>Test 2</u>	<u>Test 3</u>
Reagent B (FIB)	0.200	0.200	0.200
Reagent G (PLS)	0.125	0.125	0.125

Mix by swirling and equilibrate at 37°C for 3 minutes. Then add:

Reagent A (BUF)	0.075	0.065	0.055
Reagent I (Test)	0.050	0.060	0.070

Mix by swirling and equilibrate at 37°C for 1 minute. Then add:

Reagent F (THR)	0.050	0.050	0.050
-----------------	-------	-------	-------

Mix by swirling and incubate at 37°C for 2-3 minutes and record the initial time (T_i) in minutes after addition of Reagent F. Gently add the glass bead to each container starting with Test-1. Record time as the final time (T_f) when the glass bead touches the bottom of the container.

CALCULATIONS:

Analysis of Standard-1:

$$\Delta T (\text{Std}_1) = T_f (\text{Std}_1) - T_i (\text{Std}_1)$$

$$\text{Log } \Delta T (\text{Std}_1)$$

$$\text{Log } \frac{\text{Units}}{\text{Std Reaction Mixture}} = \text{Log } \frac{(\text{units/vial}) \times \text{DF}}{0.125}$$

units/vial = units/vial claimed by Chromogenix
 DF = Dilution factor
 MI = Milliliters of standard used in reaction mixture

Plot the log ΔT ($T_f - T_i$) versus log units of Tissue Plasminogen Activator per reaction mixture as a linear standard curve. Determine the linear equation for the standard curve.

Analysis of Test – 1

$$\frac{\text{Units}}{\text{Test Reaction Mixture}} = \frac{(\text{units liberated from Std Curve}) \times \text{DF}}{0.050}$$

Units liberated = y
 DF = Dilution factor
 0.050 = Milliliters of Test used in reaction mixture

Enzymatic Assay of Tissue Plasminogen Activator Fibrinolytic Assay

REFERENCE:

Fearnlay, Clinical Sciences, 16, pp 645-650 (1957).

Bachmann, F. and Kruithof E., Seminars in Thrombosis and Hemostasis, 10, 6 (1984).

Klauser, A. Biotechnology, 4, 706 (1986).

NOTES:

1. Product No. T-4055 is not stable when diluted. For Prod. No. T-4055, immediately before use, prepare a solution containing approximately 500 units/ml in cold Reagent E. Dilute this solution to approximately 2500 units/ml with cold Reagent A. Use solution within 5 minutes of preparation.
2. This assay is based on the cited references.

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