

Enzymatic Assay of STREPTOKINASE

PRINCIPLE:

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

Fibrin (insoluble) $\xrightarrow[\text{Complex}]{\text{Plasmin Streptokinase}}$ Fibrin Fragments (soluble)

CONDITIONS: T = 37°C, pH 7.5

METHOD: Visual Determination of Clot Liquification

REAGENTS:

- A. 197 mM Borate/Borax Buffer with 0.9% (w/v) Sodium Chloride, pH 7.5 at 37EC
(Borate Buffer)
(Prepare 100 ml in deionized water using 1.11 gram of Boric Acid, Sigma Prod. No. B-0252, 0.7 gram of Borax, Sigma Prod. No. B-9876, and 0.9 g of Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 7.5 at 37°C with either 1 M HCl or 1 M NaOH.)
- B. 9.0% (w/v) Fibrinogen Solution (Fibrinogen)¹
(Prepare 10 ml in Reagent A using Fibrinogen, Sigma Prod. No. F-8630. To solubilize place in a 37°C water bath for 1-2 hours.)
- C. Plasminogen Solution (Plasminogen)
(Prepare approximately 10.0 ml of a solution containing 1.7 units/ml in Reagent A using Plasminogen, Sigma Prod. No. P-5661. Store on ice.)
- D. Thrombin Solution
(Prepare 2.5 ml of a solution containing 100 units/ml in Reagent A using Thrombin, Sigma Prod. No. T-6884. Store on ice.)

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REAGENTS: (continued)

- E. 0.50% (w/v) Gelatin Solution with 100 mM Potassium Phosphate, 0.1% (w/v) Sodium Chloride, and 0.01% (w/v) Thimerosal (Gelatin Diluent)
(Prepare 100 ml in deionized water using Gelatin, Sigma Prod. No. G-2500, Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, Sodium Chloride, Sigma Prod. No. S-9625, and Thimerosal, Sigma Prod. No. T-5125.)
- F. Streptokinase Standard (Std)
(Immediately before use, prepare a solution containing approximately 175 units/ml Streptokinase in Reagent E. World Health Organization (WHO) Standards for Streptokinase or a WHO traceable lot should be used as the standard.)
- G. Streptokinase Solution
(Immediately before use, prepare a solution containing approximately 175 units/ml Streptokinase in Reagent E.)
- H. 4 mm Glass Beads (Glass Bead)
(Use 4 mm Glass Bead, Fisher Scientific Catalog No. 11-312B.)

PROCEDURE:

A. Clot Formation Check

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

	<u>Test</u>
Reagent A (Borate Buffer)	0.250
Reagent E (Gelatin Diluent)	0.150
Reagent B (Fibrinogen)	0.400
Reagent C (Plasminogen)	0.250

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

Reagent D (Thrombin)	0.100
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Mix by swirling and incubate at 37°C for approximately 2-3 minutes to allow for clot formation. 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, 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.

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B. Clot Lysing Check

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

	<u>Test</u>
Reagent A (Borate Buffer)	0.250
Reagent E (Gelatin Diluent)	0.100
Reagent B (Fibrinogen)	0.400
Reagent C (Plasminogen)	0.250

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

Reagent F (STD)	0.050
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Mix by swirling, equilibrate at 37°C for 1 minute. Then add:

Reagent D (Thrombin)	0.100
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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 formation. 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 the 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.

C. Standard Curve

Pipette (in milliliters) the following reagents into a suitable container:²

	<u>Std1</u>	<u>Std2</u>	<u>Std3</u>	<u>Std4</u>	<u>Std5</u>	<u>Std6</u>
Reagent A (Borate Buffer)	0.250	0.250	0.250	0.250	0.250	0.250
Reagent E (Gelatin Diluent)	0.145	0.140	0.138	0.135	0.130	0.120
Reagent B (Fibrinogen)	0.400	0.400	0.400	0.400	0.400	0.400
Reagent C (Plasminogen)	0.250	0.250	0.250	0.250	0.250	0.250

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

Reagent F (STD)	0.005	0.010	0.012	0.015	0.020	0.030
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Mix by swirling, equilibrate at 37°C for 1 minute. Then add:

Reagent D (Thrombin)	0.100	0.100	0.100	0.100	0.100	0.100
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C. Standard Curve (continued)

Mix by swirling and incubate at 37°C for 2-3 minutes and record the initial time (T_i) in minute after addition of Reagent D. 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.

D. Test

Pipette (in milliliters) the following reagents into a suitable container³:

	<u>S1</u>	<u>S2</u>	<u>S3</u>	<u>S4</u>
Reagent A (Borate Buffer)	0.250	0.250	0.250	0.250
Reagent E (Geltain Diluent)	0.140	0.138	0.135	0.130
Reagent B (Fibrinogen)	0.400	0.400	0.400	0.400
Reagent C (Plasminogen)	0.250	0.250	0.250	0.250

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

Reagent G (Streptokinase Soln)	0.010	0.012	0.015	0.020
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Equilibrate at 37°C for 1 minute. Then add:

Reagent D (Thrombin)	0.100	0.100	0.100	0.100
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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 Sample 1. Record time as the final time (T_f) when the glass bead touches the bottom of the container.

CALCULATIONS:

Standard Curve:

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

Plot the Log (ΔT) of the Standards vs the Log of the units of Streptokinase per reaction mixture, [Log(Units/ml)].

Sample Determination:

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

Calculate the Log (ΔT) of the sample and use the standard curve to determine the Log of the units/ml of Streptokinase in the reaction mixture [Log(units/ml)].

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CALCULATIONS: (continued)

Units/ml = {Antilog[Log(units/ml)Streptokinase from Curve]}(DF) Enzyme

df = Dilution factor

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

UNIT DEFINITION:

One unit will liquify a standard clot of fibrinogen, plasminogen and thrombin at pH 7.5 at 37°C in 10 minutes.

FINAL ASSAY CONCENTRATION:

In a 1.15 ml reaction mix, the final concentrations are 171 mM borate, 0.065% (w/v) gelatin, 3.1% (w/v) fibrinogen, 0.43 unit plasminogen, 0.88-5.3 units streptokinase, 10 units thrombin, 0.79% (w/v) sodium chloride, 0.001% (w/v) thimerosal, and 13 mM potassium phosphate.

NOTES:

1. The concentration of Fibrinogen required will vary from lot to lot. This solution should be yellow in color. It may require filtering before use.
2. Due to the variances in the concentration of fibrinogen used in the assay, it may be required to add additional higher or lower standards to the standard curve in order to obtain reasonable clot lysing times. Clot lysing times for the standard curve are generally between 7 and 45 minutes.
3. Due to variances in the standard curve, altering the amount of sample aliquot used in the assay may be required.
4. 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.