

Enzymatic Assay of PLASMINOGEN

PROCEDURE:

Plasminogen $\xrightarrow{\text{Urokinase}}$ Plasmin

a-Casein (Perchloric Acid Insoluble) $\xrightarrow{\text{Plasmin}}$ Perchloric Acid Soluble
Casein Fragments

CONDITIONS: T = 37°C, pH = 7.5, A_{275nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 400 mM Potassium Phosphate Buffer, pH 7.5 at 37°C (Phosphate Buffer)
(Prepare 350 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 37°C with 1 M KOH.)
- B. 10 mM Potassium Phosphate with 70 mM Sodium Phosphate and 100 mM Lysine, pH 7.5 at 37°C (Phosphate/Lysine Buffer)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876, and L-Lysine, Monohydrochloride, Sigma Prod. No. L-5626. Adjust to pH 7.5 at 37°C with either 1 M HCl or 1 M NaOH.)
- C. 1% (w/v) Perchloric Acid Solution (Perchloric Acid)
(Prepare 1000 ml by diluting 14.3 ml of Perchloric Acid, Sigma Stock No. 24,425-2, to 1000 ml with deionized water.)

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REAGENT: (continued)

- D. 3% (w/v) a-Casein Solution (a-Casein)
(Prepare 330 ml by dispersing 10 g of a-Casein, Sigma Prod. No. C-7891, in 85 ml of deionized water. Add 7 ml of 1 M NaOH and heat on a hot plate until the a-Casein is dissolved. Then cool the solution and adjust the volume to 700 ml using deionized water. Add 3 ml of 1 M HCl to the solution with stirring and then add an additional 13 ml of 1 M HCl. Slowly transfer the a-Casein solution to 1000 ml of Reagent C (Perchloric Acid) with stirring. Allow the solution to stand overnight at room temperature. Centrifuge the resulting suspension at room temperature. Wash the precipitate three times with 150 ml of deionized water and suspend the washed precipitate in 120 ml of Phosphate Buffer (Reagent A). Adjust the pH of the suspension to 7.5 using 1 M NaOH and stir until the a-Casein is completely dissolved. Readjust to pH 7.5 and dilute to 330 ml using Phosphate Buffer (Reagent A). Freeze in aliquots of 5 to 10 ml. The substrate is **very unstable**; keep frozen until ready to use. Thaw out in a 37°C water bath and use as soon as the last ice crystals melt.)
- E. 1.7 M Perchloric Acid Solution (PA)
(Prepare 100 ml in deionized water using Perchloric Acid, Sigma Stock No. 24,425-2.)
- F. Urokinase Enzyme Solution (Urokinase)
(Use Urokinase, Sigma Prod. No. U-8627, undiluted, approximately 25 units/ml.)
- G. Plasminogen Solution
(Immediately before use, prepare a solution containing 1.0 unit/ml of Plasminogen in cold deionized water.)

PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Phosphate/Lysine Buffer)		0.50
		0.50
Reagent G (Plasminogen)		0.30
		0.30

Reagent F (Urokinase)	0.01	-----
Deionized Water	0.20	0.20

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PROCEDURE: (continued)

Mix by swirling and incubate at 37°C for 15 minutes. Then add:

Reagent D (a-Casein)	1.00	1.00
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Mix by swirling and incubate at 37°C for 20 minutes. Then add:

Reagent E (PA)	3.00	3.00
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Mix by swirling. Then add:

Reagent F (Urokinase)	-----	0.01
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Mix by swirling and allow to stand at 25°C for 20 minutes. Centrifuge both the Test and Blank solutions. Then filter through Whatman #54 filter paper. If the solutions are still hazy, filter through a 0.45 µm syringe filter. Transfer the filtered solutions to suitable cuvettes and record the $A_{275\text{nm}}$ for both the Test and Blank. The corrected absorbance for the Test should be below 0.5.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(A_{275\text{nm}} \text{ Test} - A_{275\text{nm}} \text{ Blank}) (df)}{(0.3)(1)}$$

df = Dilution factor

0.3 = Volume (in milliliter) of plasminogen

1 = Change in absorbance at $A_{275\text{nm}}$ as per the Unit Definition

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

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

UNIT DEFINITION:

One unit will produce a ΔA_{275} of 1.0 from a-casein in 20 minutes at pH 7.5 at 37°C, when measuring perchloric acid soluble products in a volume of 5.0 ml. Activity is determined after activation to plasmin with urokinase.

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FINAL ASSAY CONCENTRATIONS:

In a 2.01 ml reaction mix, the final concentrations are 17 mM sodium phosphate, 202 mM potassium phosphate, 25 mM L-lysine, 1.5% (w/v) a-casein, 0.25 unit urokinase, and 0.3 unit plasminogen.

REFERENCE:

Hedner, U., Nilsson, I.M., and Robertson, B. (1966) *Thromb. Diath. Haemorrhag.* **16**, 38-50

Lauritsen, O.S. (1966) *Scandinavian Journal of Clinical and Laboratory Investigation* **18**, 73-79

Lauritsen, O.S. (1966) *Scandinavian Journal of Clinical and Laboratory Investigation* **18**, 69-72

Lauritsen, O.S. (1969) *Scandinavian Journal of Clinical and Laboratory Investigation* **23**, 121-128

Lauritsen, O.S. (1968) *Scandinavian Journal of Clinical and Laboratory Investigation* **22**, 239-246

NOTES:

1. Varying volumes of Plasminogen (not exceeding 0.50 ml) may be added to the test reaction mix. Bring the volume of plasminogen up to 0.50 ml with deionized water. For each test a blank must be run containing the same amount of plasminogen.
2. This assay is based on the cited references.
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.