

**Enzymatic Assay of PLASMIN
(EC 3.4.21.7)**

PRINCIPAL:

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. 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 1 M NaOH.)
- B. 400 mM Sodium Phosphate Buffer, pH 7.5 at 37°C (Phosphate Buffer)
(Prepare 350 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.5 at 37°C using 1 M NaOH.)
- C. 1% (w/v) Perchloric Acid Solution
(Prepare 1000 ml by diluting 14.3 ml of Perchloric Acid, Sigma Stock No. 24,425-2, to 1000 ml with deionized water.)
- 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 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 13 ml more. Slowly transfer the Casein solution to 1000 ml of 1% Perchloric Acid (Reagent C) with stirring. Allow the solution to stand overnight)

at room temperature. Centrifuge the resulting suspension at low speed the next day.)

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REAGENTS: (continued)

- D. Wash the precipitate three times with 150 ml each of deionized water and suspend the washed precipitate in 120 ml of Phosphate Buffer (Reagent B). Adjust the pH of the suspension to 7.5 using 1 M NaOH and stir until the Casein is completely dissolved. Readjust the pH to 7.5 and dilute to 330 ml using Phosphate Buffer (Reagent B). Freeze in aliquots of 5 - 10 ml. This 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 (Perchloric Acid) (Prepare 100 ml in deionized water using Perchloric Acid, Sigma Stock No. 24,425-2.)
- F. Plasmin Solution (Plasmin) (Immediately before use prepare a solution containing 1 unit/ml Plasmin in cold deionized water.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable tubes.

	<u>Test</u>	<u>Blank</u>
Reagent A (Phosphate Lysine Buffer)		0.50 0.50
Reagent F (Plasmin) ¹	0.30	-----
Deionized Water	0.20	0.20

Mix by swirling and then add:

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

Reagent E (Perchloric Acid)	3.00	3.00
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Mix by swirling gently. Then add:

Reagent F (Plasmin)	-----	0.30
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Mix by swirling and allow to stand at 25°C for 20 minutes.

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PROCEDURE: (continued)

Centrifuge and then filter the solutions through #54 Whatman filter paper. If the solutions are still hazy, filter through 0.45 µm syringe filters. Transfer the solutions to suitable cuvettes and record the $A_{275\text{nm}}$ for both the Test and Blank. The absorbance for the blank should be between 0.075 and 0.10. The corrected absorbance for both the samples and controls should be below 0.50.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(A_{275\text{nm}} \text{ Test} - A_{275\text{nm}} \text{ Blank})(\text{df})}{(0.30)(1)}$$

df = Dilution factor

0.30 = Volume (in milliliter) of enzyme used

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 $\Delta A_{275\text{nm}}$ 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.

FINAL ASSAY CONCENTRATIONS:

In a 2.00 ml reaction mix, the final concentrations are 2.5 mM potassium phosphate, 1.5% (w/v) a-casein, 218 mM sodium phosphate, 25 mM lysine, and 0.3 unit plasmin.

REFERENCES:

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

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

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NOTES:

1. Varying volumes of plasmin (not exceeding 0.50 ml) may be added to the test reaction mix. Bring the volume of plasmin up to 0.50 ml with deionized water. For each test a blank must be run containing the same amount of plasmin.
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.