SIGMA QUALITY CONTROL TEST PROCEDURE

**ProductInformation**

**Enzymatic Assay of UROKINASE**
**(EC 3.4.21.73)**

**PRINCIPLE:**

Plasminogen + $H_2O$ $\xrightarrow{\text{Urokinase}}$ Plasmin

Casein $\xrightarrow{\text{Plasmin}}$ Perchloric Acid Soluble Amino Acids

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

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

A. 60 mM Tris HCl Buffer with 90 mM Sodium Chloride, pH 7.5 at 37°C.
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 7.5 at 37°C with 1 M HCl.)

B. Porcine Plasminogen Solution (Plas)
(Immediately before use, prepare a solution containing 2 units/ml Plasminogen, Sigma Prod. No. P-1048, in deionized water.)

C. 1.4% (w/v) α-Casein Suspension (Casein)
(Prepare 100 ml in Reagent A using α-Casein, Sigma Prod. No. C-7891. Do not heat. Stir to make a homogenous suspension.)

D. 500 mM Perchloric Acid Reagent (PCA)
(Prepare 100 ml in deionized water using Perchloric Acid, Sigma Stock No. 24425-2.)

E. Urokinase Enzyme Solution
(Immediately before use, prepare a solution containing 1 - 2 units/ml of Urokinase in cold Reagent A.)

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**PROCEDURE:**

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

| | Test | Blank |
|---|---|---|
| Reagent A (Buffer) | 0.90 | 0.90 |
| Reagent B (Plas) | 1.00 | 1.00 |

Mix by swirling and equilibrate to 37°C. Then add:

| | | |
|---|---|---|
| Reagent E (Enzyme Solution) | 0.10 | ------ |

Immediately following enzyme addition, add:

| | | |
|---|---|---|
| Reagent C (Casein) | 2.00 | 2.00 |

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

| | | |
|---|---|---|
| Reagent D (PCA) | 6.00 | 6.00 |
| Reagent E (Enzyme Solution) | ------ | 0.10 |

Mix by swirling and incubate at 25°C for 60 minutes. Filter the solution through a Whatman #50 filter paper[1] and transfer the filtrate to suitable quartz cuvettes. Record the $A_{275nm}$ for both the Test and Blank using a suitable spectrophotometer.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(A_{275nm}\ \text{Test} - A_{275nm}\ \text{Blank})(10)}{(1)(15)(0.1)}$$

10 = Volume (in milliliters) of assay
1 = Change in Absorbance as per the Unit Definition
15 = Time of reaction (in minutes) as per the Unit Definition
0.1 = Volume (in milliliters) of enzyme used

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

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

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**UNIT DEFINITION:**

One unit will activate that amount of porcine plasminogen which will produce a $\Delta A_{275nm}$ of 1.0 per ml per minute at pH 7.5 at 37°C, when measuring perchloric acid soluble products from α-casein (1 cm light path).[2]

**FINAL ASSAY CONCENTRATION:**

In a 4 ml reaction mix, the final concentrations are 45 mM Tris, 68 mM sodium chloride, 2 units plasminogen, 0.70% (w/v) α-casein and 0.1 - 0.2 unit urokinase.

**REFERENCES:**


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. Filtering the solution by vacuum or with 0.45 µm syringe filters will result in lower activity.
2. Higher activities are obtained when human plasminogen is used as a substrate.
3. This assay is based on the cited references.
4. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.