Enzymatic Assay of UROKINASE
(EC 3.4.21.73)

PRINCIPLE:

Plasminogen + H₂O Urokinase → Plasmin

Casein Plasmin → Perchloric Acid Soluble Amino Acids

CONDITIONS:  T = 37°C, pH = 7.5, A₂₇₅nm, 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) a-Casein Suspension (Casein)
   (Prepare 100 ml in Reagent A using a-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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Reagent B (Plas)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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

<p>| | | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately following enzyme addition, add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Casein)</td>
<td>2.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (PCA)</td>
<td>6.00</td>
<td>6.00</td>
</tr>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by swirling and incubate at 25°C for 60 minutes. Filter the solution through a Whatman #50 filter paper and transfer the filtrate to suitable quartz cuvettes. Record the A\textsubscript{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 $A_{275nm}$ of 1.0 per ml per minute at pH 7.5 at 37°C, when measuring perchloric acid soluble products from a-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) a-casein and 0.1 - 0.2 unit urokinase.

REFERENCES:


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.

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