Urokinase
Determination of urokinase activity with a chromogenic substrate

Measurement Principle
The urokinase activity is determined by its amidolytic effect on the substrate pyro-Glu-Gly-Arg-pNA (S-2444). The rate at which p-nitroaniline (pNA) is released is measured photometrically at 405 nm. This can be followed on a recorder (initial rate method) or read after stopping the reaction with acetic acid (acid stopped method). The correlation between DA/min (or absorbance) and the urokinase activity is linear in the range 5–40 Ploug or CTA units. The urokinase concentration should preferably be given in units of substrate hydrolysing activity, but may be calculated by using standards prepared from a standard urokinase preparation. The amidolytic activity, however, does not necessarily parallel the fibrinolytic activity for different urokinases.

\[
\text{pGlu-Gly-Arg-pNA + H}_2\text{O} \rightarrow \text{Urokinase} \rightarrow \text{pGlu-Gly-Arg-OH + pNA}
\]

Reagents

1. CS-61(44), 25 mg    Art. No. 229061
   Reconstitute the substrate with 16.7 ml of distilled water.
2. Urokinase standard
   The urokinase standard is dissolved in or diluted with Solvent (Reagent 3) to a concentration of 400 units/ml (Ploug or CTA units). The dilution is stable for one day at 2-8°C.
3. Solvent
   Distilled water containing 5 g/l of Carbowax 6000 (Union Carbide, NY, USA).
4. Tris Buffer, pH 8.8 (25°C)
   Tris 6.1 g (50 mmol/l)
   NaCl 2.2 g (38 mmol/l)
   Distilled water 800 ml
   Adjust the pH to 8.8 at 25°C by adding an appropriate amount (approx. 12 ml) of 1 mol/l HCl. Fill up to 1000 ml with distilled water. The buffer, if not contaminated, will remain stable for two months at 2-8°C. Note: Although the substrate is quite selective, there may be a risk for influence of other proteases if the preparation is heavily contaminated. The addition of Trasylol (aprotinin), 10 KIU/ml, to the buffer may in such cases be favourable.
5. Acetic acid 20% or citric acid 2%; acid is used in the acid-stopped method.

Sample
The urokinase is dissolved in or diluted with Solvent (Reagent 3) to a concentration of approximately 400 units/ml (Ploug or CTA units) By using commercially available urokinase (Leo or Abbott) it was found that the dilution was stable for at least one day when kept at 2-8°C. Note: if the urokinase preparation is contaminated with proteolytic enzymes, Trasylol (aprotinin) may be added to a concentration of 10 KIU/ml in order to increase the stability.

Standardisation
40 units: Use the urokinase standard 400 units/ml (Reagent 2). 5 units: Use the urokinase standard 400 units/ml (Reagent 2) diluted 1:8 with buffer (Reagent 4).

Standard curve
The urokinase standard 400 units/ml (Reagent 2) is further diluted according to the table below:
**Method**

### Initial rate method

<table>
<thead>
<tr>
<th>Buffer</th>
<th>800 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubate at 37°C</td>
<td>5-10 min</td>
</tr>
<tr>
<td>Urokinase samples/standards</td>
<td>100 µl</td>
</tr>
<tr>
<td>Incubate at 37°C</td>
<td>1-2 min</td>
</tr>
<tr>
<td>Substrate (37°C)</td>
<td>100 µl</td>
</tr>
<tr>
<td>Mix</td>
<td></td>
</tr>
</tbody>
</table>

Transfer sample immediately to a 1 cm semi-microcuvette (preheated to 37°C) for measurement of the absorbance change in a photometer at 405 nm and at 37°C, calculate ΔA/min.

### Acid stopped method

<table>
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<td>Acetic acid or citric acid</td>
<td>100 µl</td>
</tr>
<tr>
<td>Mix</td>
<td>yes</td>
</tr>
</tbody>
</table>

Incubate at 37°C 5 min

Read the absorbance (A) of the sample against a water or sample blank in a photometer at 405 nm. The colour is stable for at least 4 hours.

### Calculation

Plot ΔA/min or A for the standards against their known urokinase activity. Calculate the urokinase activity of the sample in Ploug or CTA units. By multiplying the results with 10 the concentration in units/ml is obtained. The urokinase activity can also be calculated from the following formulas:

**Initial rate method:**

\[
\mu\text{kat/l} = \Delta A/\text{min} \times 17.4 \\
\text{U/l} = \Delta A/\text{min} \times 1042
\]

**Acid stopped method:**

\[
\mu\text{kat/l} = A \times 3.8 \\
\text{U/l} = A \times 229
\]

### Bibliography