UROKINASE

Determination of urokinase activity with S-2444.

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 ΔA/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.

$$pGlu\text{-}Gly\text{-}Arg\text{-}pNA + H_2O \xrightarrow{Urokinase} pGlu\text{-}Gly\text{-}Arg\text{-}OH + pNA$$

Reagents

1. S-2444, 25 mg Art. No. S820357
Reconstitute the substrate S-2444 (MW: 498.9) 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%
Acetic acid is used in the acid-stopped method.

Equipment

1. Spectro- or filter photometer, 405 nm
2. Semi-microcuvettes, 1 cm.
3. Thermostat, 37°C
4. Stop watch
5. Disposable plastic tubes
6. Photometer with cuvette housing, thermostated at 37°C (for the initial rate 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:

Ploug or CTA Units	Urokinase standard (400 units/ml) µl	Solvent µl
5	100	700
10	100	300
20	200	200
30	300	100
40	400	-

Method

Initial rate method	
Buffer	800 µl
Incubate at 37°C	5-10 min
Urokinase sample/standards	100 µl
Mix and incubate at 37°C	1-2 min
Substrate (37°C)	100 µl

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	Sample	Sample blank
Buffer	800 µl	800 µl
Incubate at 37°C	5-10 min	-
Urokinase sample/standards	100 µl	100 µl
Mix and incubate at 37°C	1-2 min	-
Substrate (37°C)	100 µl	-
Mix and incubate at 37°C	5 min	-
Acetic acid 20%	100 µl	100 µl
Mix	yes	yes
Substrate (37°C)	-	100 µl
Mix	-	yes

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: $\mu kat/l = \Delta A/min \times 17.4$
$U/l = \Delta A/min \times 1042$

Acid stopped method: $\mu kat/l = A \times 3.8$
$U/l = A \times 229$

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