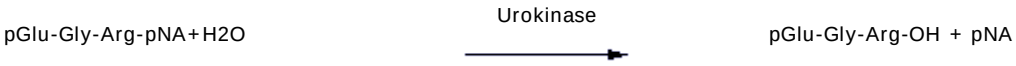
Urokinase

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. 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.



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:

Plong or CTA Units	Urokinase standard (400 units/ml)	Solvent
	µl	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 samples/standards	100 µl
Incubate at 37°C	1-2 min
Substrate (37°C)	100 µl
Mix	

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 samples/standards	100 µl	100 µl
Incubate at 37°C	1-2 min	-
Substrate (37°C)	100 µl	-
Incubate at 37°C	5 min	-
Acetic acid or citric acid	100 µl	100 µl
Mix	yes	yes
Incubate at 37°C	5 min	-
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:
µkat/l = ΔA/min x 17.4
U/l = ΔA/min x 1042

Acid stopped method:
µkat/l = A x 3.8
U/l = A x 229

Bibliography

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