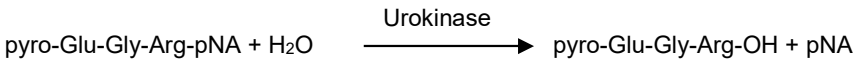
Standardisation of Urokinase

Method Sheet

Principle



The urokinase activity is determined by its amydolytic 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 △A/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 splitting activity, but may also 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.

Equipment

1. Spectro- or filterphotometer, 405 nm
2. Semi-microcuvettes, 1 cm
3. Waterbath or thermostate, 37°C
4. Stop-watch
5. Disposable plastic tubes

Additional equipment for the initial rate method:

6. Photometer with cuvette housing, thermostated at 37°C
7. Recorder

Reagents

1. Substrate S-2444 (pyro-Glu-Gly-Arg-pNA•HCl), mol. wt. 498.9 Art. No. 82 03 57

Each vial contains 25 mg S-2444. The substance is dissolved in 16.7 ml of distilled water. If prepared with sterile water and not contaminated by micro-organisms, the 3 mmol/l solution is stable for at least six months at 2-8°C.

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, N.Y., USA).

4. 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 (approximately 12 ml) of 1 mol/l HCl. Fill up to 1000 ml with distilled water. The buffer, if not contaminated, is 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) (Bayer, FRG), 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.

Procedure

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) (Bayer, FRG) 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). (See Methods)
5 units:	Use the urokinase standard 400 units/ml (Reagent 2) diluted 1:8 with buffer (Reagent 4). (See Methods)
Standard curve:	See appendix.

Methods

Initial rate method		Sample
Buffer	µl	800
Thermostate at 37°C (5-10 min)		X
Urokinase sample or urokinase standards	µl	100
Mix and thermostate at 37°C (1-2 min)		X
S-2444 (37°C)	µl	100
Mix		X

Transfer the 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 △A/min.

Acid stopped method		Sample	Sample blank
Buffer	µl	800	800
Thermostate at 37°C (5-10 min)		X	-
Urokinase sample or urokinase standards	µl	100	100
Mix and thermostate at 37°C (1-2 min)		X	-
S-2444 (37°C)	µl	100	-
Mix and incubate		300	-
Acetic acid 20%	µl	100	100
Mix immediately		X	X
S-2444 (37°C)	µl	-	100
Mix		-	X

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 △A/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 = △A/min x 17.4
U/l = △A/min x 1042

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

References

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Appendix

Standard curve

The urokinase standard 400 units/ml (Reagent 2) is further diluted according to the table below.

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