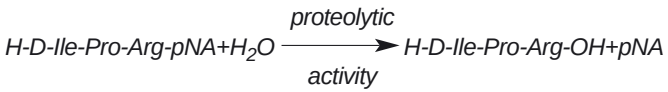
PROTEOLYTIC ACTIVITY

Determination of proteolytic activity in plasma, serum or euglobulin fractions with S-2288.

Measurement Principle

Several proteases with arginine specificity readily split the substrate H-D-Ile-Pro-Arg-pNA (S-2288). The proteolytic activity is thus determined by the rate at which p-nitroaniline (pNA) is released. The formation of pNA can be followed spectrophotometrically at 405 nm by using a recorder (initial rate method).
The correlation between the change in absorbance per minute (ΔA/min) and the proteolytic activity is usually linear in the 0.05 - 0.5 µkat/l or 3 - 30 U/l range. If possible the linearity of the assay should be checked for each individual type of sample. This can be done by serial dilution of the sample. In several instances the proteolytic activity may originate from α2-macroglobulin enzyme complexes.



Reagents

1. S-2288, 25 mg Art. No. S820852

Reconstitute the substrate S-2288 (MW: 577.6) with 7.2 ml of distilled water to obtain a 6 mmol/l solution.

2. Tris Buffer, pH 8.4 (25°C)

Tris	12.1 g	(100 mmol/l)
NaCl	6.2 g	(106 mmol/l)
Distilled Water	800 ml	

Adjust the pH to 8.4 at 25°C by adding an appropriate amount (approximately 44 ml) of 1 mol/l HCl.
Fill up to 1000 ml with distilled water. The buffer, if not contaminated, is stable for six months at 2-8°C.

Equipment

1. Spectro- or filter photometer, 405 nm with cuvette housing, thermostated at 37°C
2. Semi-microcuvettes, 1 cm
3. Thermostat, 37°C
4. Stop watch
5. Disposable plastic tubes

Sample

Dilute the plasma, serum or euglobulin fraction with buffer (Reagent 2) to a proteolytic activity of 0.05 - 0.5 µkat/l or 3 - 30 U/l.

Method

Initial rate method	
Buffer	**200 µl**
Incubate at 37°C	**2-4 min**
Diluted sample (20-25°C)	**200 µl**
Mix and incubate at 37°C	**2-4 min**
Substrate (37°C)	**200 µl**

Mix and 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.

Calculation

The proteolytic activity in the sample is calculated from the following formulas:
µkat/l = ΔA/min x 5.21 x F
U/l = ΔA/min x 313 x F
F = dilution factor (e.g. 10 if the sample is diluted 1:10 before initial rate determination).

Note: For some enzymes with low Km less substrate can be used.

Bibliography

Bratt G et al. Factors and inhibitors of blood coagulation and fibrinolysis in acute nonlymphoblastic leukemia. Scand J Haematol 34, 332-339 (1985).

Schoenberger OL et al. Proteolytic activity of human tumor cell lines deriving from bronchial squamous cell carcinoma, pulmonary metastasis of rhabdomyosarcoma and pleural metastasis of mesothelioma. Eur J Respir Dis 71, 434-443 (1987).

Burnouf-Radosevich M and Burnouf T. A therapeutic, highly purified factor XI concentrate from human plasma. Transfusion 32, 861-867 (1992).

Koyama S et al. Antiproteases attenuate the release of neutrophil chemotactic activity from bronchial epithelial cells induced by smoke. Exp Lung Res 22, 1-19 (1996).