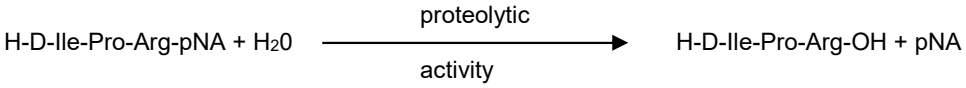
Proteolytic Activity
Method Sheet S-2288

Determination of Proteolytic Activity in Plasma, Serum or Euglobulin Fractions

Principle:



Serveral proteases with arginine specificity readily split the substrate H-D-Ile-Pro-Arg-pNA • 2HCl (S-2288).
The proteolytic acitivity is thus determined by the rate at which p-nitroaniline metrically 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 acitivity may originate from α_2-macroglobulin enzyme complexes.

Reagents:

1. Substrate S-2288 (H-D-Ile-Pro-Arg-pNA • 2HCl) mol.wt. 577.6.
S-2288 from KabiVitrum Ab, Sweden.
The substance (25 mg) is dissolved in 7.2 ml of distilled water. If prepared with sterile water and not contaminated by microorganisms, the 6 mmol/l solution is stable for at least two months at 2 to 8°C.

2. Buffer pH 8.4 (25°C)

Tris	12.1 g (100 mmol/l)
NaCl	6.2g (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 to 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

Procedure:

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.

Methods:

Initial rate method		Volume
Buffer	µl	200
Thermostate at 37°C (2-4 min)		X
Sample dilution (20-25°C)	µl	200
Mix and thermostate at 37°C (2-4 min)		X
S-2288 (37°C)	µl	200
Mix		X

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 K_m less substrate can be used.

Bibliography

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