Proteolytic Activity

Determination of proteolytic activity in plasma, serum or euglobulin fractions with CS-05(88)

Measurement Principle

Several proteases with arginine specificity readily split the substrate H-D-Ile-Pro-Arg-pNA. 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 a2-macroglobulin enzyme complexes.

HH-D-Ile-Pro-Arg-pNA + H2O $\xrightarrow{\text{proteolytic activity}}$ H-D-Ile-Pro-Arg-OH + pNA

Reagents

1. CS-05(88), 25 mg Art. No. 229091
   Reconstitute the substrate with 7.2 ml of distilled water to obtain a 6 mmol/l solution.
2. Tris-NaCl buffer, pH 8.4 Art. No. AR009A
3. HCl, 1 mmol/l for dilution of samples.

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
Incubate at 37°C	2-4 min
Substrate (37°C)	100 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

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