Trypsin

Determination of trypsin in duodenal fluid with a chromogenic substrate

Measurement Principle

Trypsin catalyses the hydrolysis of p-nitroaniline (pNA) from the substrate Bz-Ile-Glu-(OR)-Gly-Arg-pNA. The rate at which pNA is released is followed on a photometer at 405 nm. The reaction rate increases linearly with increasing activities of trypsin up to at least 4.8 µkat/l, which corresponds to a trypsin concentration of 2 mg/l.

Bz-Ile-Glu-Gly-Arg-pNA + H2O —(Trypsin)→ Bz-Ile-Glu-Gly-Arg-OH + pNA

Reagents

1. CS-11(22), 25 mg Art. No. 229015
   Reconstitute the substrate with 34 ml of distilled water.
2. Tris/Calcium Buffer, pH 8.2 (25°C)

Tris	6.1 g	(50 mmol/l)
CaCl2	2.2 g	(20 mmol/l)
Distilled water	800 ml	

Adjust the pH to 8.2 at 25°C by adding an appropriate amount of 1 mol/l HCl. Make up to 1000 ml with distilled water. If not contaminated by microorganisms, the buffer is stable for six months at 2 to 8°C.

3. HCl, 1 mmol/l is used for dilution of samples.

Sample

A single lumen plastic tube is used (ID:2 mm, OD:4 mm, length: 125 cm) with 4-6 holes cut in the distal 10 cm and a stainless leader at the tip. The position of the tube is checked by X-ray immediately before the test. Duodenal fluid is collected after stimulating pancreatic secretion with either 300 ml of water, orally, or preferably secretin, intravenously, 1U/kg body weight
Duodenal fluid is collected in 4 x 15 min samples by siphon action in 250 ml plastic bottles and kept on ice (1°C). The samples may be stored at -20°C for not more than a week. Just before analysis, thaw the sample quickly at 37°C. If the fluid is turbid, centrifuge it at 2-8°C and then keep the supernatant on ice. Determine the pH of the samples. (Note: if the pH of the duodenal fluid is below 5, this indicates the presence of a large amount of gastric juice, which may yield an incorrect value).
Dilute the sample at 1:100 or 1:1000 with 1 mmol/l HCl and keep it on ice. At low trypsin activities the sample is assayed undiluted or diluted 1:10.

Method

Initial rate method	
Buffer	800 µl
Incubate at 37°C	5-6 min
Diluted sample (20-25°C)	100 µl
Incubate at 37°C	1-2 min
Substrate (37°C)	100 µl
Mix	

Transfer the sample immediately to a 1 cm semi-microcuvette (preheated to 37°C) and measure the change in absorbance in a photometer at 405 nm and at 37°C.

Calculation

Calculate ΔA/min for the sample.
The trypsin activity is then calculated from the formula:

µkat/l = ΔA/min x 17.36 x F

U/l = ΔA/min x 1042 x F

F = Dilution factor for sample (e.g. 100 when diluted 1:100).

Bibliography

1. Bergström K & Lundh G. Determination of trypsin in duodenal fluid as a test of pancreatic function. A methodological note. Scand J Gastroent 5, 533-536, (1970).
2. Bergström K. Determination of trypsin in duodenal fluid using a new chromogenic substrate and a reaction rate instrument. LKB application note 211, March 1976.