

## Trypsin

Determination of trypsin in duodenal fluid with S-2222

### Measurement Principle

Trypsin catalyses the hydrolysis of p-nitroaniline (pNA) from the substrate Bz-Ile-Glu-(OR)-Gly-Arg-pNA (S-2222). 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  $\mu\text{kat/l}$ , which corresponds to a trypsin concentration of 2 mg/l.



### Reagents

1. S-2222, 25 mg Art. No. 82 03 16  
Reconstitute the substrate S-2222 (MW: 741.3) with 34 ml of distilled water.
2. Tris/Calcium Buffer, pH 8.2 (25°C)

Tris	6.1 g	(50 mmol/l)
CaCl <sub>2</sub>	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  
1 mmol/l HCl 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 $\mu\text{l}$
Incubate at 37°C	5-6 min
Diluted sample (20-25°C)	100 $\mu\text{l}$
Incubate at 37°C	1-2 min
Substrate (37°C)	100 $\mu\text{l}$
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  $\Delta A/\text{min}$  for the sample.

The trypsin activity is then calculated from the formula:

$$\mu\text{kat/l} = \Delta A/\text{min} \times 17.36 \times F$$

$$\text{U/l} = \Delta A/\text{min} \times 1042 \times F$$

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.