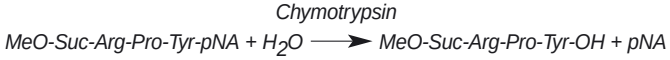
CHYMOTRYPSIN

Determination of chymotrypsin with S-2586.

Measurement Principle

The chymotrypsin activity is determined by its amidolytic effect on the substrate MeO-Suc-Arg-Pro-Tyr-pNA (S-2586). The rate at which p-nitroaniline (pNA) is released is measured photometrically at 405 nm. This can be followed on a recorder (initial rate method) or read after stopping the reaction with acetic acid (acid stopped method). The correlation between the change in absorbance per minute (ΔA/min) or absorbance (A) and the chymotrypsin activity is linear in the 0.05-1.0 µkat/l or 3-60 U/l range. The amidolytic activity of different chymotrypsin preparations does not necessarily parallel the protease activity.



Reagents

1. S-2586, 25 mg Art. No. S820894
Reconstitute the substrate S-2586 (MW: 705.3) with 60 ml of distilled water.

2. Tris/Calcium Buffer, pH 8.3 (25°C)

Tris	12.1 g	(100 mmol/l)
NaCl	56.2 g	(960 mmol/l)
Distilled water	800 ml	

Adjust the pH to 8.3 at 25°C by adding approximately 50 ml of 1 mol/l HCl. Add 10 ml of 1 mol/l $CaCl_2$ solution. Fill up to 1000 ml with distilled water. The buffer, if not contaminated, will remain stable for two months at 2-8°C.

3. Acetic acid, 20%
Acetic acid is used in the acid-stopped method.

Equipment

1. Spectro- or filter photometer, 405 nm
2. Semi-microcuvettes, 1 cm.
3. Thermostat, 37°C
4. Stop watch
5. Disposable plastic tubes
6. Photometer with cuvette housing, thermostated at 37°C (for the initial rate method)

Sample

The sample containing chymotrypsin is dissolved in or diluted with 1 mmol/l HCl to a concentration of 0.1 g/l.
This stock solution is stable for more than two weeks at 2-8°C. Before assay, the solution is diluted 1:200 with 1 mmol/l HCl.
If the sample is a pure protein, it is advisable to use 0.1% Carbowax 6000 (Union Carbide, NY) or 1% albumin (previously checked for amidolytic activity) to avoid adsorption to surfaces.

Method

Initial rate method	
Buffer	**200 µl**
Incubate at 37°C	**3-4 min**
Chymotrypsin sample	**200 µl**
Mix and incubate at 37°C	**2-3 min**
Substrate (37°C)	**200 µl**
Mix	

Transfer the 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.

Acid stopped method	Sample	Blank
Buffer	**200 µl**	**200 µl**
Incubate at 37°C	**3-4 min**	**-**
Chymotrypsin sample	**200 µl**	**200 µl**
Mix and incubate at 37°C	**2-3 min**	**-**
Substrate (37°C)	**200 µl**	**-**
Mix and incubate at 37°C	**3 min**	**-**
Acetic acid 20%	**200 µl**	**200 µl**
Mix	**yes**	**-**
Substrate (37°C)	**-**	**200 µl**
Mix	**-**	**yes**

Read the absorbance (A) of the sample against a water or sample blank in a photometer at 405 nm.
The colour is stable for at least 4 hours.

Calculation

Calculate the chymotrypsin activity of the stock solution from the following formulas:

Initial rate method:	µkat/l	=	5.19 x ΔA/min x 200
	U/l	=	311 x ΔA/min x 200
Acid stopped method:	µkat/l	=	2.31 x A x 200
	U/l	=	138 x A x 200

Bibliography

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