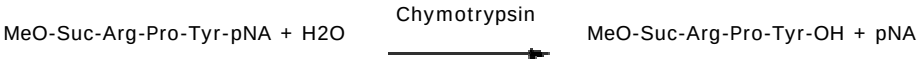
Chymotrypsin

Determination of chymotrypsin activity 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 (DA/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. 82 08 94
   Reconstitute the substrate S-2586 (MW: 705.3) with 60 ml of distilled water.
2. Buffer - Tris/Calcium, pH 8.3 (25°C)
3.

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.

4. Acetic acid 20%
   Acetic acid is used in the acid-stopped 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	200 µl

Transfer 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.

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

Read the absorbance (A) of the sample against distilled water at 405 nm within 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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