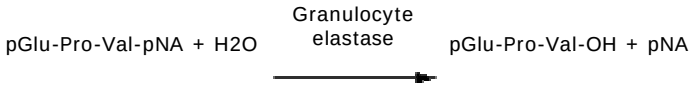
Granulocyte Elastase

Determination of granulocyte elastase activity with S-2484

Measurement Principle

The elastase activity is determined by its amidolytic effect on the substrate pyro-Glu-Pro-Val-pNA (S-2484). 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 granulocyte elastase activity is linear in the 0.1-1.5 µkat/l or 6-90 U/l range. The amidolytic activity does not necessarily parallel the elastolytic activity for different elastase preparations.



Reagents

1. S-2484, 25 mg Art. No. 82 08 86
   Reconstitute the substrate S-2484 (MW: 445.5) with 7 ml of DMSO. One volume of this stock solution is diluted with 3 volumes of distilled water.
2. Tris Buffer, pH 8.3 (25°C)

Tris	12.1 g	(100 mmol/l)
NaCl	52.6 g	(960 mmol/l)
Distilled water	800 ml	

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

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

Sample

The sample containing granulocyte elastase is dissolved in or diluted with distilled water, saline or buffer to an activity of 0.1-1.5 µkat/l which approximately corresponds to a concentration of 0.5-7.5 mg/l of a rather pure enzyme. 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
Elastase sample	200 µl
Mix and incubate at 37°C	2-3 min
Substrate	200 µl

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 DA/min.

Acid stopped method	Sample	Blank
Buffer	200 µl	200 µl
Incubate at 37°C	3-4 min	-
Elastase sample	200 µl	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	-	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 elastase activity of the sample from the formulas:

Initial rate method:
µkat/l = 5.19 x ΔA/min
U/l = 311 x ΔA/min

Acid stopped method:
µkat/l = 2.31 x A
U/l = 138 x A

Bibliography

1. Kramps JA et al. L-Pyroglutamyl-L-prolyl-L-Valine-p-nitroanilide, a highly specific substrate for granulocyte elastase. Scand J Clin Lab Invest 43, 427-432, (1983).
2. Tanaka H et al. A sensitive and specific assay for granulocyte elastase in inflammatory tissue fluid using L-pyroglutamyl-L-prolyl-L- valine-p-nitroanilide. Clin Chim Acta 187, 173-180 (1990).
3. Liu R et al. Leukocyte functions in 2 cases of Papillon-Lefevre syndrome. J Clin Periodontol 27, 69-73 (2000)