ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of ELASTASE, Leukocyte
(EC 3.4.21.37)
Sigma Prod. No. E-8140

PRINCIPLE:

N-t-BOC-Ala-p-NP $\xrightarrow{\text{Elastase}}$ N-t-BOC-Ala + p-Nitrophenol

Abbreviations used:
N-t-BOC-Ala-p-NP = N-t-BOC-L-Alanine p-Nitrophenyl Ester
N-t-BOC-Ala = N-t-BOC-L-Alanine
N-t-BOC = N-tert-Butoxy-Carbonyl

CONDITIONS: T = 37°C, pH = 6.5, $A_{347.5nm}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Sodium Phosphate, pH 6.5 at 37°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust the pH to 6.5 at 37°C with 1 M NaOH.)

B. Acetonitrile
(Use Acetonitrile, Sigma Prod. No. A-6914.)

C. 0.20 mM N-t-BOC-L-Alanine p-Nitrophenyl Ester Solution (BOC-Ala-NP)
(Prepare 51 ml by dissolving the required amount of N-t-BOC-L-Alanine p-Nitrophenyl Ester, Prod. No. 15052, in 1.0 ml of Reagent B. Dilute this solution to 50 ml with Reagent A. Adjust the pH, if necessary, to 6.5 with either 1 M NaOH or 1 M HCl, as required. **PREPARE FRESH.**)

D. Elastase Enzyme
(One 1 unit vial of Elastase, Leukocyte.)

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PROCEDURE:

Place Reagent C (BOC-Ala-NP) in a water bath and equilibrate to 37°C. Pipette (in milliliters) the following reagent into a suitable cuvette:

	Test	Blank
Reagent C (BOC-Ala-NP)	------	3.00

Equilibrate to 37°C. Monitor the $A_{347.5nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add 3.00 ml of Reagent C (BOC-Ala-NP) to the 1 unit vial of Elastase (Reagent D). Immediately mix by inversion. Transfer the solution to a cuvette in the spectrophotometer and at precisely 1.00 minute determine the $A_{347.5nm}$ of both the Test and the Blank.

CALCULATIONS:

$$\text{Units/vial} = \frac{(A_{347.5nm}\ \text{Test} - A_{347.5nm}\ \text{Blank})(3)}{(0.0055)(60)(1)}$$

3 = Total volume (in milliliters) of assay
0.0055 = Micromolar extinction coefficient[1] of p-Nitrophenol at 347.5nm
60 = Conversion factor from minutes to seconds as per the Unit Definition
1 = Vial

$$\text{Units/mg protein} = \frac{\text{units/vial}}{\text{mg protein/vial}}$$

UNIT DEFINITION:

One unit will release one nanomole of p-nitrophenol per second from N-t-BOC-L-alanine p-nitrophenyl ester at pH 6.5 at 37°C.

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FINAL ASSAY CONDITIONS:

In a 3.00 ml reaction mix, the final concentrations are 49 mM sodium phosphate, 0.20 mM N-t-BOC-L-alanine p-nitrophenyl ester, 2% (v/v) acetonitrile, and 1 unit of elastase.

REFERENCE:

Cotter, T.G. and Robinson, G.B. (1980) *Biochimica et Biophysica Acta* **615**, 414-425

Visser, L. and Blout, E.R. (1972) *Biochimica Et Biophysica Acta* **268**, 257-260

NOTES:

1. The millimolar extinction coefficient of p-nitrophenol at 347.5 nm is described in Visser, L. and Blout, E.R. (1972).

2. This assay is based on the cited reference.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.