



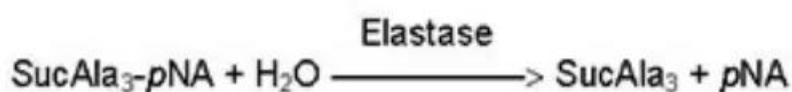
上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话: 021-61312973 传真: 021-55068248
网址: www.shyuanye.com
邮箱: shyysw@sina.com

Enzymatic Assay of Elastase

DESCRIPTION:

This procedure may be used for Elastase products using SucAla₃-pNA as the substrate.

The continuous spectrophotometric rate determination (A_{410} , Light path = 1 cm) is based on the following reaction:



where:

SucAla₃-pNA = N-Succinyl-Ala-Ala-Ala-p-nitroanilide

SucAla₃ = N-Succinyl-Ala-Ala-Ala

pNA = p-Nitroanilide

Unit Definition - One unit of Elastase will hydrolyze 1.0 μ mole of N-succinyl-L-Ala-Ala-Ala-p-nitroanilide per minute at pH 8.0 at 25 ° C.

PRECAUTIONS:

Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

REAGENTS AND EQUIPMENT REQUIRED:

Trizma base

N-Succinyl-Ala-Ala-Ala-p-nitroanilide

PREPARATION INSTRUCTIONS:

Use ultrapure water ($\geq 18 \text{ M } \Omega \text{ xcm}$ resistivity at 25 °C) for the preparation of reagents.

Buffer (100 mM Tris HCl, pH 8.0 at 25 °C) - Prepare a 12.1 mg/mL solution of Trizma base in ultrapure water. Adjust the pH to 8.0 at 25 °C with 1 M HCl.

Substrate Solution (4.4 mM SucAla₃-pNA Solution) -Prepare 2 mg/mL solution of N-Succinyl-Ala-Ala-Ala-p-nitroanilide in Buffer.



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Enzyme Solution (Elastase)-Immediately before use, prepare a solution containing 0.2 – 0.5 unit/mL of Elastase in cold (2-8 °C) buffer.

PROCEDURE:

In a 3.00 mL reaction mix, the final concentrations are 96.7 mM Trizma, 0.29 mM N-Succinyl-Ala-Ala-Ala-*p*-nitroanilide, and 0.02 – 0.05 unit of Elastase.

1. Pipette the following reagents into suitable cuvettes:

Reagent	Test (mL)	Blank (mL)
Buffer	2.70	2.80
Substrate Solution	0.20	0.20

2. Mix by inversion and equilibrate to 25 °C. Then add:

Reagent	Test (mL)	Blank (mL)
Enzyme Solution	0.10	—

3. Immediately mix by inversion and record the increase in A_{410} for ~5 minutes. Obtain the $\Delta A_{410}/\text{minute}$ using the maximum linear rate for both the Test and Blank using a minimum of 4 data points over a one minute time interval.

RESULTS:

Calculations

1.

$$\text{Units/mL enzyme} = \frac{(\Delta A_{410}/\text{min Test} - \Delta A_{410}/\text{min Blank}) (3.00) (\text{df})}{(8.8) (0.10)}$$

where:

3.00 = Total volume (mL) of assay

df = Dilution factor

8.8 = Millimolar extinction coefficient of *p*-Nitroaniline at 410 nm at pH 8.0

0.1 = Volume (mL) of Enzyme Solution used

2.

$$\text{Units/mg solid} = \frac{\text{units/mL enzyme}}{\text{mg solid/mL enzyme}}$$