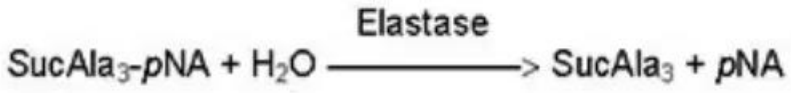
Enzymatic Assay of Elastase

DESCRIPTION：

This procedure may be used for Elastase products using SucAla$_3$-*p*NA as the substrate.

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



where:

SucAla$_3$-*p*NA = N-Succinyl-Ala-Ala-Ala-$_p$-nitroanilide

SucAla$_3$ = N-Succinyl-Ala-Ala-Ala

*p*NA = *p*-Nitroanilide

Unit Definition － One unit of Elastase will hydrolyze 1.0 µmole 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 (≥18 MΩxcm resistivity at 25 ℃) for the preparation of reagents.

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

Substrate Solution (4.4 mM SucAla$_3$-*p*NA Solution) -Prepare 2 mg/mL solution of N-Succinyl-Ala-Ala-Ala-*p*-nitroanilide in Buffer.

Enzyme Solution (Elastase)-Immediately before use, prepare a solution containing 0.2 – 0.5 unit/mL of Elastase in cold (2-8 ℃) 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 ℃. 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 ΔA_{410}/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.

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

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.

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