

**SIGMA QUALITY CONTROL TEST
PROCEDURE****Enzymatic Assay of ELASTASE
(EC 3.4.21.36)****PRINCIPLE:**

Abbreviations Used:

SucAla₃-PNA = N-Succinyl-ALA-ALA-ALA p-NitroanilideSucAla₃ = N-Succinyl-ALA-ALA-ALA

PNA = p-Nitroaniline

CONDITIONS: T = 25°C, pH = 8.0, A_{410nm}, Light path = 1 cm**METHOD:** Continuous Spectrophotometric Rate Determination**REAGENTS:**

- A. 100 mM Tris HCl Buffer, pH 8.0 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 25°C with 1 M HCl.)
- B. 4.4 mM SucAla₃-PNA Solution (SucAla₃-PNA)
(Prepare 5 ml in Reagent A using N-Succinyl-ALA-ALA-ALA p-Nitroanilide, Sigma Prod. No. S-4760.)
- C. Elastase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2-0.5 units/ml of Elastase in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.70	2.80
Reagent B (SucAla ₃ -PNA)	0.20	0.20

Enzymatic Assay of ELASTASE (EC 3.4.21.36)

REAGENTS: (continued)

Mix by inversion and equilibrate to 25°C using a suitably thermostatted spectrophotometer. Then add:

	<u>Test</u>	<u>Blank</u>
Reagent C (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the increase in $A_{410\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{410\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{410\text{nm}} \text{ Test} - \Delta A_{410\text{nm}} \text{ Blank})(3)(df)}{(8.8)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 micromole of succinyl-ala-ala-ala p-nitroanilide per minute at pH 8.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix the final concentrations are 100 mM Tris, 0.29 mM N-succinyl-ALA-ALA-ALA p-nitroanilide and 0.02-0.05 unit elastase.

**Enzymatic Assay of ELASTASE
(EC 3.4.21.36)**

REFERENCE:

Bieth, J., Spiess, B., and Wermuth, C.G. (1974) *Biochemical Medicine* **11**, 350-357

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

Sigma warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.