SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of PROTEASE
(EC 3.4.21.19)
Sigma Prod. Nos. P-2922, P-6306, and P-8400

PRINCIPLE:

N-t-BOC-GAPE + H2O $\xrightarrow{\text{Protease}}$ N-t-BOC-L-Glutamic Acid + Phenol

Abbreviations:
BOC = N-tert-Butoxy-Carbonyl
N-t-BOC-GAPE = N-t-BOC-L-Glutamic Acid á-Phenyl Ester

CONDITIONS: T = 37°C, pH = 7.8 A_{270nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Tris-Phosphate Buffer, pH 7.8 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.8 at 37°C with Phosphoric Acid, Sigma Prod. No. P-6560.)

B. 60 mM N-t-BOC-L-Glutamic Acid á-Phenyl Ester Substrate Solution (N-t-BOC-GAPE)
(Prepare by dissolving 97 mg of N-t-BOC-L-Glutamic Acid á-Phenyl Ester, Sigma Prod. No. B-3016, in 5 ml of 1,4-Dioxane, Sigma Prod. No. D-9553. Make sure that the solid is at room temperature before opening. Any moisture in this substrate will cause immediate hydrolysis. **PREPARE FRESH**.)

C. Protease Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 2.0 units/ml of Protease in deionized water.)

Enzymatic Assay of PROTEASE
(EC 3.4.21.19)
Sigma Prod. Nos. P-2922, P-6306, and P-8400

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.85	2.85
Reagent B (N-t-BOC-GAPE)	0.05	0.05

Mix by inversion and equilibrate to 37°C. Monitor the A_{270nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (Enzyme Solution)	0.10	------
Deionized Water	------	0.10

Immediately mix by inversion and record the increase in A_{270nm} for approximately 5 minutes. Obtain the ΔA_{270nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{270nm}/\text{min Test} - \Delta A_{270nm}/\text{min Blank})(3)(df)}{(1.5)(0.1)}$$

3 = Volume (in milliliters) of assay
df = Dilution factor
1.5 = Millimolar extinction coefficient of phenol at 270 nm
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 ìmole of N-t-BOC-L-glutamic acid á-phenyl ester per minute at pH 7.8 at 37°C. One unit is equivalent to approximately 0.004 casein digestion unit.

Enzymatic Assay of PROTEASE
(EC 3.4.21.19)
Sigma Prod Nos. P-2922, P-6306, and P-8400

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 190 mM Tris, 1 mM N-t-BOC-L-glutamic acid á-phenyl ester, 1.7% 1,4-dioxane and 0.01 - 0.2 unit protease.

REFERENCE:

Drapeau, G. R. (1976) *Methods of Enzymology* **45**, 469.

NOTES:

1. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.