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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of TRYPSIN[1] (EC 3.4.21.4)

PRINCIPLE:

BAEE + H_2O $\xrightarrow{\text{Trypsin}}$ Nα-Benzoyl-L-Arginine + Ethanol

Abbreviation used:
BAEE = Nα-Benzoyl-L-Arginine Ethyl Ester

CONDITIONS: T = 25EC, pH = 7.6, A_{253nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 67 mM Sodium Phosphate Buffer, pH 7.6 at 25EC
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.6 at 25EC with 1 M NaOH.)

B. 0.25 mM Nα-Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)
(Prepare 50 ml in Reagent A using Nα-Benzoyl-L-Arginine Ethyl Ester, Hydrochloride, Sigma Prod. No. B-4500.)

C. 1 mM Hydrochloric Acid Solution (HCl)
(Prepare 50 ml in deionized water using concentrated Hydrochloric Acid, Sigma Prod. No. H-7020.)

D. Trypsin Enzyme Solution
(Immediately before use, prepare a solution containing 500 BAEE units/ml of Trypsin in cold Reagent C.)

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PROCEDURE:

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

	Test	Blank
Reagent B (BAEE)	3.00	3.00

Equilibrate to 25EC. Monitor the A_{253nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (Hcl)	------	0.20
Reagent D (Enzyme Solution)	0.20	------

Immediately mix by inversion and record the increase in A_{253nm} for approximately 5 minutes. Obtain the ΔA_{253nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{BAEE Units/ml enzyme} = \frac{(\Delta A_{253nm}/\text{min Test} - \Delta A_{253nm}/\text{min Blank})(df)}{(0.001)\ (0.20)}$$

df = Dilution factor
0.001 = The change in A_{253nm}/minute per unit of Trypsin at pH 7.6 at 25EC in a 3.2 ml reaction mix
0.20 = Volume (in milliliters) 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 BAEE unit will produce a ΔA_{253nm} of 0.001 per minute with BAEE as substrate at pH 7.6 at 25EC in a reaction volume of 3.2 ml.

FINAL ASSAY CONCENTRATION:

In a 3.2 ml reaction mix, the final concentrations are 63 mM sodium phosphate, 0.23 mM Nα-benzoyl-L-arginine ethyl ester, 0.06 mM hydrochloric acid, and 100 units trypsin.

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REFERENCE:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed) Volume I, 2nd ed., 515-516, Academic Press, Inc.,New York, NY

NOTES:

1. This assay procedure is not to be used to assay Sigma Prod. Nos. T-1763, T-4019, T-8386, T-8899, and T-9906.

2. This assay is based on the cited reference.

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