



Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of TRYPSIN¹ (EC 3.4.21.4)

PRINCIPLE:



Abbreviation used:

BAEE = N α -Benzoyl-L-Arginine Ethyl Ester

CONDITIONS: T = 25°C, 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 25°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.6 at 25°C with 1 M NaOH.)
- B. 0.25 mM N α -Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)
(Prepare 50 ml in Reagent A using N α -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:

| | <u>Test</u> | <u>Blank</u> |
|------------------|-------------|--------------|
| Reagent B (BAEE) | 3.00 | 3.00 |

Equilibrate to 25°C. Monitor the $A_{253\text{nm}}$ 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 $A_{253\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{253\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

df = Dilution factor

0.001 = The change in $A_{253\text{nm}}/\text{minute}$ per unit of Trypsin at pH 7.6 at 25°C 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 $\Delta A_{253\text{nm}}$ of 0.001 per minute with BAEE as substrate at pH 7.6 at 25°C 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 N α -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.

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