SIGMA QUALITY CONTROL TEST
PROCEDURE

Enzymatic Assay of TRYSIN
(EC 3.4.21.4)

PRINCIPLE:

BAEE + \( H_2O \xrightarrow{\text{Trypsin}} N\alpha-\text{Benzoyl-L-Arginine} + \text{Ethanol} \)

Abbreviation used:
BAEE = \( N\alpha\)-Benzoyl-L-Arginine Ethyl Ester

CONDITIONS: \( T = 25^\circ C, \text{pH} = 7.6, A_{253nm}, \text{Light path} = 1 \text{ cm} \)

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 \( N\alpha\)-Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)
(Prepare 50 ml in Reagent A using \( N\alpha\)-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.)
Enzymatic Assay of TRYSIN
(EC 3.4.21.4)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (BAEE)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Equilibrate to 25°C. Monitor the $A_{253\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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<tbody>
<tr>
<td>Reagent C (Hcl)</td>
<td>-----</td>
<td>0.20</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.20</td>
<td>-----</td>
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</tbody>
</table>

Immediately mix by inversion and record the increase in $A_{253\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{253\text{nm}}$/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 $A_{253\text{nm}}$/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 Nα-benzoyl-L-arginine ethyl ester, 0.06 mM hydrochloric acid, and 100 units trypsin.
Enzymatic Assay of TRYPsin\textsuperscript{1}
(EC 3.4.21.4)

REFERENCE:


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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