Enzymatic Assay of TRYPSIN TPCK/DITC GLASS
(EC 3.4.21.4)

PRINCIPLE:

\[ \text{BAEE} + \text{H}_2\text{O} \xrightarrow{\text{Trypsin}} \text{Na-Benzoyl-}L\text{-Arginine} + \text{Ethanol} \]

Abbreviation used:
BAEE = Na-Benzoyl-\text{-Arginine Ethyl Ester}

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

METHOD: Spectrophotometric Rate Determination

REAGENTS:

A. 400 mM Sodium Phosphate Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous.
Adjust to pH 7.6 with 1 M HCl.)

B. 0.26 mM Na-Benzoyl-\text{-Arginine Ethyl Ester Solution (BAEE)}
(Prepare 50 ml in Reagent A using Na-Benzoyl-\text{-Arginine Ethyl Ester, Hydrochloride.})

C. Trypsin Enzyme Standard Solution (STD)
(Immediately before use, prepare a solution containing 0.01 mg/ml of Trypsin, in cold Reagent A.)

D. Trypsin Insoluble Enzyme (Trypsin/DITC)
(Use Trypsin, TPCK Bound to DITC glass.)

PROCEDURE:

Step 1: Standard Determination

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

<table>
<thead>
<tr>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (BAEE)</td>
<td>3.10</td>
</tr>
</tbody>
</table>
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**PROCEDURE:**  (continued)

Equilibrate to 25°C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Std)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>--------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and incubate at 25°C for exactly 1.5 minutes. Record the $A_{253\text{nm}}$ for the Standard and Blank using a suitably thermostatted spectrophotometer.

**Step 2: Sample Determination**

Weigh (in milligrams) the following reagent into a suitable container.

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (Trypsin/DITC)</td>
<td>5-10</td>
<td>------</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent B (BAEE)</td>
<td>3.10</td>
<td>3.10</td>
</tr>
</tbody>
</table>

Immediately vortex at a medium speed for exactly 1.5 minutes. Then filter the Test and Blank through a 0.45 µm syringe filter. Record the $A_{253\text{nm}}$ for both the Test and Blank filtrate using a suitable spectrophotometer.

**CALCULATION:**

Standard:

$$\text{Units/ml enzyme} = \frac{(A_{253\text{nm}} \text{ Test} - A_{253\text{nm}} \text{ Blank})(df)}{(0.001)(1.5)}$$

$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 mixture

1.5 = Time (in minutes) of assay
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CALCULATION:  (continued)

\[
\frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}} \times \frac{\text{mg solid}}{\text{Units/mg solid}}
\]

Compare the activity of the standard to its release value (activity). This is to ensure that the assay is functioning correctly.

Test:

\[
\text{Units/g enzyme} = \frac{(A_{253\text{nm}} \text{ Test} - A_{253\text{nm}} \text{ Blank}) \times 1000}{(0.001)(1.5)(1000)}
\]

1000 = Conversion from mg to g
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
1.5 = Time (in minutes) of assay

UNIT DEFINITION:

One BAEE unit will produce a \(A_{253\text{nm}}\) 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 400 mM sodium phosphate, 0.25 mM Na-benzoyl-L-arginine ethyl ester, 5 – 10 mg of trypsin, TPCK bound to DITC glass.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.
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This procedure is for informational purposes.