SIGMA QUALITY CONTROL TEST

Enzymatic Assay of TRYPsin INHIBITOR

PRINCIPLE:

Trypsin

\[
BAEE + H_2O \rightarrow \alpha\text{-Benzoyl-L-Arginine + Ethanol}
\]

Abbreviation used:

BAEE = N\alpha\text{-Benzyol-L-Arginine Ethyl Ester}

CONDITIONS: 

T = 25°C, pH = 7.6, A_{25\text{nm}}, 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\alpha\text{-Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)}
   (Prepare 50 ml in Reagent A using N\alpha\text{-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 (Trypsin)
   (Immediately before use, prepare a solution containing 1 mg protein/ml of Trypsin, Prod. No. T-8003, in cold Reagent C.)

E. Trypsin Inhibitor Solution (Inhib)
   (Immediately before use, prepare a solution containing 1.0 mg/ml of Trypsin Inhibitor in cold Reagent A.2,3)
Enzymatic Assay of TRYPsin INHIBITOR

PROCEDURE:

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

Part A:

<table>
<thead>
<tr>
<th>Uninh</th>
<th>Test1</th>
<th>Test2</th>
<th>Test3</th>
<th>Test4</th>
<th>Test5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (Inhib)</td>
<td>---</td>
<td>0.10</td>
<td>0.15</td>
<td>0.20</td>
<td>0.25</td>
</tr>
<tr>
<td>Reagent D (Trypsin)</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Allow to stand at 25°C for a minimum of five minutes and no longer than six minutes.

Mix by inversion and pipette (in milliliters) the following reagents into suitable cuvettes:

Part B:

<table>
<thead>
<tr>
<th>Uninh</th>
<th>Test1</th>
<th>Test2</th>
<th>Test3</th>
<th>Test4</th>
<th>Test5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (BAEE)</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Reagent C (HCl)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the \( A_{253nm} \) until constant, using a suitably thermostatted spectrophotometer. Then add:

| Uninh (Part A) | 0.10 | --- | --- | --- | --- | --- |
| Test 1 (Part A) | --- | 0.10 | --- | --- | --- | --- |
| Test 2 (Part A) | --- | --- | 0.10 | --- | --- | --- |
| Test 3 (Part A) | --- | --- | --- | 0.10 | --- | --- |
| Test 4 (Part A) | --- | --- | --- | --- | 0.10 | --- |
| Test 5 (Part A) | --- | --- | --- | --- | --- | 0.10 |

Immediately mix by inversion and record the increase in \( A_{253nm} \) for approximately 5 minutes. Obtain the \( \Delta A_{253nm} \)/minute using the maximum linear rate for the Tests, Blank, and Uninhibited Solution.

CALCULATIONS:

Trypsin Activity in BAEE units/ml enzyme =

\[
\frac{(\Delta A_{253nm}/\text{min Test} - \Delta A_{253nm}/\text{min Blank})(df)(10.0)}{(0.001)(0.10)(0.5)}
\]

\( df = \) Dilution factor
0.001 = The change in \( A_{253nm} \)/minute per unit of Trypsin at pH 7.6 at 25°C in a 3.2 ml reaction mix
0.10 = Volume (in milliliters) enzyme used (Part B)
10.0 = Total volume in milliliters of assay (Part A)
0.5 = Volume (in milliliters) of enzyme used (Part A)
Enzymatic Assay of TRYP SIN INHIBITOR

CALCULATIONS: (continued)

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

Plot the Trypsin activity (BAEE units/mg protein) vs ml of Trypsin Inhibitor/RM

Mg Trypsin Inhibitor = (ml of Trypsin Inhibitor)(Conc. of Trypsin Inhibitor, mg/ml)

Mg Trypsin Inhibited by 1 mg Trypsin Inhibitor = mg Trypsin/RM (normalizing factor)

\[
= \frac{\text{mg Trypsin Inhibitor (normalizing factor)}}{\text{mg Trypsin Inhibitor (from plot)}}
\]

Normalizing Factor = (BAEE Units of Uninhibited Trypsin per mg solid/10,000 BAEE units of Trypsin per specification.)

UNIT DEFINITION:

One trypsin 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 3.20 ml reaction mix, the final concentrations are 63 mM sodium phosphate, 0.23 mM BAEE, 0.002 mM HCl, 0.005 mg trypsin, and 0.0003 - 0.001 mg trypsin inhibitor.

NOTES:


2. When assaying Trypsin Inhibitor, Ovoinhibitor, Prod. No. T-1886, the diluent used is 200 mM sodium phosphate, monobasic, pH 7.6 at 25°C.

3. When assaying Trypsin Inhibitor, Type II-S, Sigma Prod. No. T-9128, prepare a solution containing 0.60 mg/ml of Trypsin Inhibitor in cold Reagent A.

4. The uninhibited Trypsin activity should be within 85% of the release value for activity. With a 11,700 to 13,005 Trypsin units/mg solid per label, the acceptable range for activity of the uninhibited Trypsin reaction should be 10,000 to 15,300 Trypsin units/mg solid. This range should also correspond to a corrected \( \Delta A_{253\text{nm}} \)/minute of 0.0545 to 0.0835. With this rate and an inhibition of 20% to 80% the \( \Delta A_{253\text{nm}} \)/minute should be above the spectrophotometric rate detection limit of 0.0020.
4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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