Enzymatic Assay of CHYMOPAPAIN
(EC 3.4.22.6)

PRINCIPLE:

\[ \text{BAEE} + \text{H}_2\text{O} \overset{\text{Chymopapain}}{\rightarrow} \text{Na-Benzoyl-L-Arginine} + \text{Ethanol} \]

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

CONDITIONS: \( T = 25^\circ\text{C}, \text{pH} = 6.2 \)

METHOD: Titrimetric

REAGENTS:

A. 80 mM Na-Benzoyl-L-Arginine Ethyl Ester (BAEE)
(Prepare 100 ml in deionized water using Na-Benzoyl-L-Arginine Ethyl Ester, Hydrochloride, Sigma Prod. No. B-4500.)

B. 20 mM Ethylenediaminetetraacetic Acid (EDTA)
(Prepare 100 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)

C. 50 mM L-Cysteine Solution, pH 6.2 at 25°C (Cys)
(Prepare 50 ml in Reagent B using L-Cysteine, Hydrochloride, Monohydrate Sigma Prod. No. C-7880. Adjust to pH 6.2 with 1 M NaOH.)

D. 3.0 M Sodium Chloride Solution (NaCl)
(Prepare 50 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)

E. 100 mM Sodium Hydroxide Solution—Standardized (NaOH)
(Prepare 50 ml in cold deionized water using Sodium Hydroxide, Anhydrous, Sigma Stock No. 505-8. Standardize according to the ACS Reagent Procedure.)*

F. Chymopapain Enzyme Solution
(Immediately before use, prepare a solution containing 0.75 - 1.5 units/ml of Chymopapain in deionized water.)
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PROCEDURE:

Pipette (in milliliters) the following reagents into a suitable container:

Test

<table>
<thead>
<tr>
<th>Reagent A (BAEE)</th>
<th>7.00</th>
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</thead>
<tbody>
<tr>
<td>Reagent C (Cys)</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent D (NaCl)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mix by swirling and equilibrate to 25°C. Adjust the pH to 6.3 at 25°C with 0.1 M NaOH. Then add:

| Reagent F (Enzyme Solution) | 1.00 |

When the pH drops to 6.2, begin timing the reaction. Run the reaction for 1 - 5 minutes. Maintain the pH of the reaction mix at pH 6.2 by the addition of small volumes (0.025 - 0.050 ml) of Reagent E. Record the volume of Reagent E used to maintain the pH at 6.2 and the time required.

CALCULATION:

\[
\text{Units/ml enzyme} = \frac{\text{(molarity of NaOH)}(\text{NaOH})(1000)(\text{df})}{(T)(1)}
\]

NaOH = Volume (in milliliters) of Reagent E used to maintain the pH at 6.2
1000 = Conversion factor to micromoles from millimoles as per the Unit Definition
T = Time (in minutes) required to maintain the pH at 6.2
l = 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 unit will hydrolyze 1.0 µmole of BAEE to Nα-benzoyl-L-arginine per minute at pH 6.2 at 25°C.
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INITIAL ASSAY CONCENTRATIONS:

In a 10.00 ml reaction mix, the initial concentrations are 56 mM Na-benzoyl-l-arginine ethyl ester, 5 mM l-cysteine, 2 mM ethylenediaminetetraacetic acid, 300 mM sodium chloride and 0.75 - 1.5 units chymopapain.

REFERENCE:


NOTES:


2. This assay is based on the cited references.

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

This procedure is for informational purposes. For a current copy of Sigma’s quality control procedure contact our Technical Service Department.