Enzymatic Assay of ENTEROKINASE
(EC 3.4.21.9)

PRINCIPLE:

Step 1:
Trypsinogen + H₂O Enterokinase > Trypsin + Val-Asp-Asp-Asp-Asp-Lys

Step 2:
BAEE + H₂O Trypsin > Na-Benzoyl-L-Arginine + EtOH

Abbreviations used:
BAEE = Na-Benzoyl-L-Arginine Ethyl Ester
EtOH = Ethanol

CONDITIONS:  T = 25°C, pH = 5.6, A₂₅₃nm, Light path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 40 mM Succinate Buffer, pH 5.6 at 25°C
(Prepare 100 ml in deionized water using Succinic Acid, Free Acid, Sigma Prod. No. S-7501. Adjust to pH 5.6 at 25°C with 1 M NaOH.)

B. 1 mM Hydrochloric Acid with 5 mM Calcium Chloride Solution
(Prepare 100 ml in deionized water using Hydrochloric Acid, 1.0 N, Sigma Stock No. 920-1, and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)

C. 0.1% (w/v) Trypsinogen Solution (Trypsinogen)
(Immediately before use, prepare 25 ml in cold Reagent B using Trypsinogen, Sigma Prod. No. T-1143.)

D. Enterokinase Enzyme Solution
(Immediately before use, prepare a solution containing 2 - 5 units/ml of Enterokinase in cold deionized water.)
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REAGENTS: (continued)

E. 67 mM Sodium Phosphate Buffer, pH 7.6 at 25°C
(Prepare 1 liter 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.)

F. 0.248 mM Na-Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)
(Prepare 100 ml in Reagent E using Na-Benzoyl-L-Arginine Ethyl Ester, Hydrochloride, Sigma Prod. No. B-4500. PREPARE FRESH.)

G. 40 mM Hydrochloric Acid with 5 mM Calcium Chloride Solution (HCl-CaCl₂)
(Prepare 1 liter in deionized water using Hydrochloric Acid, 1.0 N, Sigma Stock No. 920-1, and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)

PROCEDURE:

Step 1:

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

<table>
<thead>
<tr>
<th></th>
<th>Test Mix</th>
<th>Blank Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.80</td>
<td>1.80</td>
</tr>
<tr>
<td>Reagent C (Trypsinogen)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Then add:

| Reagent D (Enterokinase) | 0.10     |
| Deionized Water          | --       |
| 0.10                     |

Immediately mix by inversion and incubate at 25°C for exactly 15 minutes. Then add:

| Reagent G (HCl-CaCl₂) | 3.00     |

Step 2:
Pipette (in milliliters) the following reagents into suitable quartz cuvettes:

| Reagent F (BAEE) | 3.00 | 3.00 |
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PROCEDURE: (continued)

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Mix (Step 1)</td>
<td>0.20</td>
</tr>
<tr>
<td>Blank Mix (Step 1)</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in $A_{253nm}$ for approximately 5 minutes. Obtain the $r_{A_{253nm}}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(r_{A_{253nm}}/\text{min Test} - r_{A_{253nm}}/\text{min Blank})(5.4)(\text{df})}{(0.001)(0.20 \text{ ml})(\text{P.A.})(0.024)(15)(0.1)}$$

- $5.4 = \text{Volume (in milliliters) of Step 1}$
- $\text{df} = \text{Dilution factor}$
- $0.001 = \text{The change in } A_{253nm}/\text{minute per unit of Trypsin as per the Unit Definition}$
- $0.20 = \text{Volume (in milliliter) from Step 1 used in Step 2}$
- $\text{P.A.} = \text{Potential activity of Trypsinogen}^1$
- $0.024 = \text{mg trypsin/nanomole trypsin}$
- $15 = \text{Time (in minutes) for Step 1 as per the Unit Definition}$
- $0.1 = \text{Volume (in milliliter) of enterokinase used}$

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

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will produce 1.0 nanomole of trypsin from trypsinogen per minute at pH 5.6 at 25°C.$^2$
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FINAL ASSAY CONCENTRATION:

In a 2.40 ml reaction mix, the final concentrations are 30 mM succinate, 1 mM calcium chloride, 0.2 mM hydrochloric acid, 0.5 mg trypsinogen and 0.2 - 0.5 unit enterokinase.

REFERENCES:


Baratti, J., Maroux, S. Louvard, D., and Desnuelle, P. (1973) Biochimica et Biophysica Acta 315, 147-161

NOTES:

1. The potential activity is a reported value found on the product label of Trypsinogen. THIS VALUE IS LOT SPECIFIC.

2. This unit corresponds to approximately 2.7 units of the assay at 5°C. One unit would activate 0.065 mg of trypsinogen per hour at pH 5.8 at 5°C.

3. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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