SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of ENTEROKINASE
(EC 3.4.21.9)

PRINCIPLE:

Step 1:

Trypsinogen + H_2O $\xrightarrow{\text{Enterokinase}}$ Trypsin + Val-Asp-Asp-Asp-Asp-Lys

Step 2:

BAEE + H_2O $\xrightarrow{\text{Trypsin}}$ Nα-Benzoyl-L-Arginine + EtOH

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

CONDITIONS: T = 25°C, pH = 5.6, A_{253nm}, 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.)

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 N_{α}-Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)
(Prepare 100 ml in Reagent E using N_{α}-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-$CaCl_2$)
(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:

	Test Mix	Blank Mix
Reagent A (Buffer)	1.80	1.80
Reagent C (Trypsinogen)	0.50	0.50

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-$CaCl_2$)	3.00	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:

	Test	Blank
Test Mix (Step 1)	0.20	------
Blank Mix (Step 1)	------	0.20

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

CALCULATION:

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

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

Grant, D.A.W. and Hermon-Taylor, J. (1975) *Biochem. J.* **147**, 363-366

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.