



Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

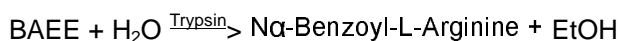
Enzymatic Assay of ENTEROKINASE (EC 3.4.21.9)

PRINCIPLE:

Step 1:



Step 2:



Abbreviations used:

BAEE = N α -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.)

**Enzymatic Assay of ENTEROKINASE
(EC 3.4.21.9)**

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-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:

	<u>Test Mix</u>	<u>Blank Mix</u>
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
------------------	------	------

**Enzymatic Assay of ENTEROKINASE
(EC 3.4.21.9)**

PROCEDURE: (continued)

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

	<u>Test</u>	<u>Blank</u>
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 $\Delta A_{253nm}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATION:

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

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 Trypsinogen¹

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

**Enzymatic Assay of ENTEROKINASE
(EC 3.4.21.9)**

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.

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.