

Enzymatic Assay of TRYPSIN-CHYMOTRYPSIN INHIBITOR Trypsin Inhibitory Activity

PRINCIPLE:



Abbreviation used:

BAEE = N α -Benzoyl-L-Arginine Ethyl Ester

This reaction is inhibited by Trypsin-Chymotrypsin Inhibitor

CONDITIONS: T = 25°C, pH = 7.6, A_{253nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 400 mM Sodium Phosphate Buffer, pH 7.6 at 25°C (Buffer I)
(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 α -Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)
(Prepare 50 ml in Reagent A using N α -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. 5 mM Sodium Phosphate Buffer, pH 7.6 at 25°C (Buffer II)
(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.)
- E. Trypsin Enzyme Solution (Trypsin)
(Immediately before use, prepare a solution containing 2.0 mg/ml of Trypsin, Sigma Prod. No. T-8253, in cold Reagent C.)
- F. Trypsin-Chymotrypsin Inhibitor Solution (Inhib)
(Immediately before use, prepare a solution containing 1.0 mg/ml of Trypsin-Chymotrypsin Inhibitor in cold Reagent D.)

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PROCEDURE:

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

Part A:

| | <u>Uninh</u> | <u>Test1</u> | <u>Test2</u> | <u>Test3</u> | <u>Test4</u> | <u>Test5</u> |
|-----------------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Reagent F (Inhib) | --- | 0.05 | 0.075 | 0.10 | 0.15 | 0.20 |
| Reagent E (Trypsin) | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Reagent D (Buffer II) | 9.50 | 9.45 | 9.425 | 9.40 | 9.35 | 9.30 |

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

Part B:

| | <u>Uninh</u> | <u>Test1</u> | <u>Test2</u> | <u>Test3</u> | <u>Test4</u> | <u>Test5</u> | <u>Blank</u> |
|-----------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Reagent B (BAEE) | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Reagent D (Buffer II) | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.20 |

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 $\Delta A_{253nm}/\text{minute}$ using the maximum linear rate for the Tests, Blank, and Uninhibited Solutions.

CALCULATIONS:

$$\text{BAEE units/ml} = \frac{(\Delta A_{256nm}/\text{min Test} - \Delta A_{256nm}/\text{min Blank})(0.5)(df)}{(0.001)(0.1)(10)}$$

0.5 = Volume (in milliliter) of enzyme used in Part A of the Assay
df = Dilution factor

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CALCULATIONS: (continued)

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 (as per the Unit Definition)

0.1 = Volume (in milliliter) of enzyme from Part A used in Part B of the Assay

10 = Volume (in milliliters) of Part A of the Assay

Plot the Trypsin activity (BAEE units/ml) versus volume (in milliliter) of Trypsin-Chymotrypsin Inhibitor/Reaction Mixture in Step A.

Determine the point at which the line intercepts the abscissa. This point is the X-intercept.

Y_0 = Mg of Trypsin-Chymotrypsin Inhibitor/ml in Part B of the Assay that results in complete inhibition of Trypsin:

$$= (X\text{-Intercept})(\text{mg TCI/ml in Reagent F})$$

CT = Mg of Trypsin/ml Part B of the Assay

$$= (0.5)(\text{mg Trypsin/ml in Reagent E})$$

Mg of Trypsin inhibited by 1 mg Trypsin-chymotrypsin Inhibitor

$$= \frac{CT}{Y_0}$$

UNIT DEFINITION:

One trypsin unit will produce a ΔA_{253nm} of 0.001 per minute with BAEE as substrate at pH 7.6 at 25°C in a reaction volume of 3.2 ml.

SPECIFICATION:

One mg protein will inhibit 3 - 5 mg of Trypsin with an activity of approximately 10,000 BAEE units per mg protein.

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FINAL ASSAY CONCENTRATIONS:

In a 3.20 ml reaction mix, the final concentrations are 375 mM sodium phosphate, 0.23 mM N α -benzoyl-L-arginine ethyl ester, 1.6 μ M HCl, 10 μ g trypsin, and 0.5 - 2.0 μ g trypsin-chymotrypsin inhibitor.

REFERENCE:

Bergmeyer, H.V., Gawehn, K. and Grassl, M. (1974) in *Methods of Enzymatic Analysis*, Volume I, 515-516, Academic Press Inc., New York, NY

Birk, Y. and Gertler, A. (1968) *Biochemical Preparations* **12**, 25-29

NOTES:

1. This assay is based on the cited references.
2. 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.