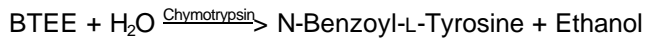


## Enzymatic Assay of TRYPSIN-CHYMOTRYPSIN INHIBITOR Chymotrypsin Inhibition

### PRINCIPLE:



Abbreviation used:

BTEE = N-Benzoyl-L-Tyrosine Ethyl Ester

This reaction is inhibited by Trypsin-Chymotrypsin Inhibitor

**CONDITIONS:** T = 25°C, pH = 7.8,  $A_{256\text{nm}}$ , Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

### REAGENTS:

- A. 80 mM Tris HCl Buffer, pH 7.8 at 25°C (Buffer I)  
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.8 at 25°C with 1 M HCl.)
- B. 50% (v/v) Methanol  
(Prepare 50 ml in deionized water using Methanol, Absolute, Sigma Stock No. 17-5.)
- C. 1.18 mM N-Benzoyl-L-Tyrosine Ethyl Ester Solution (BTEE)  
(Prepare 50 ml in Reagent B using N-Benzoyl-L-Tyrosine Ethyl Ester, Sigma Prod. No. B-6125.)
- D. 2 M Calcium Chloride Solution ( $\text{CaCl}_2$ )  
(Prepare 5 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)
- E. 1 mM Hydrochloric Acid Solution (HCl)  
(Prepare 50 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)
- F. 10 mM Tris HCl Buffer, pH 7.8 at 25°C (Buffer II)  
(Prepare 25 ml in deionized water using Reagent A. Adjust to pH 7.8 at 25°C with either 0.1 M NaOH or 0.1 M HCl.)

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**REAGENT:** (continued)

- G. Chymotrypsin Enzyme Solution (Chymo)  
(Immediately before use, prepare a solution containing 2 mg/ml of  $\alpha$ -Chymotrypsin, Sigma Prod. No. C-4129 in cold Reagent E.)
- H. Trypsin-Chymotrypsin Inhibitor Solution (TCI)  
(Immediately before use, prepare a solution containing 1 mg/ml of Trypsin-Chymotrypsin Inhibitor in cold Reagent F.)

**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 H (TCI)	---	0.05	0.075	0.10	0.15	0.20
Reagent G (Chymo)	0.50	0.50	0.50	0.50	0.50	0.50
Reagent F (BufferII)	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 C (BTEE)	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Reagent A (BufferI) 1.42	1.42	1.42	1.42	1.42	1.42	1.42	1.42
Reagent D (CaCl <sub>2</sub> )	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Reagent F (BufferII) ---	---	---	---	---	---	0.10	---

Mix by inversion and equilibrate to 25°C. Monitor the  $A_{256nm}$  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	---

## Enzymatic Assay of TRYPSIN-CHYMOTRYPSIN INHIBITOR Chymotrypsin Inhibition

### PROCEDURE: (continued)

Immediately mix by inversion and record the increase in  $A_{256\text{nm}}$  for approximately 5 minutes. Obtain the  $\Delta A_{256\text{nm}}/\text{minute}$  using the maximum linear rate for the Tests, Blank, and Uninhibited Solution.

### CALCULATIONS:

$$\text{BTEE units/ml} = \frac{(\Delta A_{256\text{nm}}/\text{min Test} - \Delta A_{256\text{nm}}/\text{min Blank})(3)(0.5)(\text{df})}{(0.964)(0.1)(10)}$$

3 = Volume (in milliliters) of Part B of the Assay

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

df = Dilution factor

0.964 = Millimolar extinction coefficient of N-Benzoyl-L-Tyrosine at 256 nm

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 Chymotrypsin activity (BTEE 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 Chymotrypsin:

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

CT = Mg of Chymotrypsin/ml in Part B of the Assay

$$= (0.5)(\text{mg Chymotrypsin/ml in Reagent G})$$

Mg of Chymotrypsin inhibited by 1 mg Trypsin-chymotrypsin Inhibitor

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

**Enzymatic Assay of TRYPSIN-CHYMOTRYPSIN INHIBITOR  
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**UNIT DEFINITION:**

One Chymotrypsin unit will hydrolyze 1.0  $\mu$ mole of BTEE per minute at pH 7.8 at 25°C.

**SPECIFICATIONS:**

One mg of protein will inhibit 2-5 mg of chymotrypsin with activity of approximately 40 BTEE units per mg protein.

**FINAL ASSAY CONCENTRATION:**

In a 3.00 ml reaction mix, the final concentrations are 38 mM Tris, 0.55 mM N-benzoyl-L-tyrosine ethyl ester, 23% (v/v) methanol, 53 mM calcium chloride, 1.7  $\mu$ M hydrochloric acid, 3.3  $\mu$ g chymotrypsin, and 0.17-0.67  $\mu$ g trypsin-chymotrypsin inhibitor.

**REFERENCES:**

Wirnt, R. (1974) in *Method of Enzymatic Analysis*, 2nd ed., Volume II, 1009-1012, 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.**