

**Enzymatic Assay of α -1-ANTITRYPSIN
Inhibition of Trypsin Activity**

PRINCIPLE:



Abbreviation used:

BAEE = Na-Benzoyl-L-Arginine Ethyl Ester

α -1-Antitrypsin inhibits this reaction.

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

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 67 mM Sodium Phosphate Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876. Adjust to pH 7.6 at 25°C with 1 M HCl.)
- B. 0.25 mM Na-Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)
(Prepare 50 ml in Reagent A using Na-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. Trypsin Enzyme Solution (Trypsin)
(Immediately before use, prepare a solution containing 0.5 mg/ml of Trypsin, Sigma Prod. No. T-8003, in cold Reagent C.)
- E. α -1-Antitrypsin Solution (α -1-Antitryp)
(Immediately before use, prepare a solution containing 2 mg solid/ml of α -1-Antitrypsin in cold Reagent A for A-9024. A-6150 requires a concentration of 4 mgs solid/ml.)

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PROCEDURE:

Step 1:

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

	<u>Uninh</u>	<u>Test 1</u>	<u>Test 2</u>	<u>Test 3</u>	<u>Test 4</u>	<u>Test 5</u>
Reagent E (α -1-Antitryp)	---	0.10	0.20	0.30	0.40	0.50
Reagent D (Trypsin)	0.50	0.50	0.50	0.50	0.50	0.50
Reagent C (HCl)	9.50	9.40	9.30	9.20	9.10	9.00

Mix by inversion.

Step 2:

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

	<u>Uninh</u>	<u>Test 1</u>	<u>Test 2</u>	<u>Test 3</u>	<u>Test 4</u>	<u>Test 5</u>	<u>Blank</u>
Reagent B (BAEE)	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Reagent C (HCl)	---	---	---	---	---	---	0.20

Mix by inversion and equilibrate to 25°C. Monitor the A_{253nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

	<u>Uninh</u>	<u>Test 1</u>	<u>Test 2</u>	<u>Test 3</u>	<u>Test 4</u>	<u>Test 5</u>	<u>Blank</u>
Uninh (Step 1)	0.20	---	---	---	---	---	---
Test 1 (Step 1)	---	0.20	---	---	---	---	---
Test 2 (Step 1)	---	---	0.20	---	---	---	---
Test 3 (Step 1)	---	---	---	0.20	---	---	---
Test 4 (Step 1)	---	---	---	---	0.20	---	---
Test 5 (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 the Tests, Blank, and Uninhibited Solution.

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CALCULATIONS:

Trypsin Activity:

$$\text{BAEE units/mg enzyme} = \frac{(A_{253\text{nm}}/\text{min Test} - A_{253\text{nm}}/\text{min Blank})(\text{df})}{(0.001)(\text{mg Trypsin/RM})}$$

df = Dilution factor

0.001 = The change in $A_{253\text{nm}}$ /minute per unit of Trypsin as per the Unit Definition (One BAEE unit = A_{253} of 0.001 per minute with BAEE as substrate at pH 7.6 at 25°C. Reaction volume = 3.2 ml (1 cm light path))

RM = Reaction Mixture

Plot the Trypsin activity versus ml of a-1-Antitrypsin. Determine from the plot the amount of a-1-Antitrypsin needed for 100% inhibition.

The amount (in mg) of a-1-Antitrypsin needed for 100% inhibition = (ml of a-1-Antitrypsin for 100% inhibition)(conc. of a-1-Antitrypsin (mg/ml))

$$\text{mg a-1-Antitrypsin to inhibit 1 mg Trypsin} = \frac{\text{mg of a-1-Antitrypsin needed for 100\% Inhibition}}{\text{mg Trypsin/Reaction Mix}}$$

FINAL ASSAY CONCENTRATIONS:

In a 3.20 reaction mix, the final concentrations are 0.23 mM Na-benzoyl-L-arginine ethyl ester, 63 mM sodium phosphate, 0.06 mM hydrochloric acid, 0.005 mg trypsin, 0.004 - 0.02 mg a-1-antitrypsin.

REFERENCE:

Bergmeyer, H.U., Gawehn, K. and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Volume I, 2nd ed., 516-517, Academic Press, New York, NY

Crawford, I.P. (1973) *Archives of Biochemistry and Biophysics* **156**, 215-222

Eriksson, S. (1965) *Acta Medica Scandinavica*, Suppementum 432, 6-12

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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.