SIGMA QUALITY CONTROL TEST PROCEDURE

**ProductInformation**

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

**PRINCIPLE:**

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

Abbreviation used:
BAEE = Nα-Benzoyl-L-Arginine Ethyl Ester

α-1-Antitrypsin inhibits this reaction.

**CONDITIONS:** T = 25°C, pH = 7.6, $A_{253nm}$, 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 Nα-Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)
(Prepare 50 ml in Reagent A using Nα-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-Antityp)
(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:

| | Uninh | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 |
|---|---|---|---|---|---|---|
| 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:

| | Uninh | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 | Blank |
|---|---|---|---|---|---|---|---|
| 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:

| | Uninh | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 | Blank |
|---|---|---|---|---|---|---|---|
| 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 $\Delta A_{253nm}$/minute using the maximum linear rate for the Tests, Blank, and Uninhibited Solution.

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**CALCULATIONS:**

Trypsin Activity:

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

df = Dilution factor
0.001 = The change in $A_{253nm}$/minute per unit of Trypsin as per the Unit Definition (One BAEE unit = $\Delta A_{253}$ 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 α-1-Antitrypsin. Determine from the plot the amount of α-1-Antitrypsin needed for 100% inhibition.

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

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

**FINAL ASSAY CONCENTRATIONS:**

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

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