SIGMA QUALITY CONTROL TEST

ProductInformation

Enzymatic Assay of TRYPSIN INHIBITOR[1]

PRINCIPLE:

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

Abbreviation used:
BAEE = N□-Benzoyl-L-Arginine Ethyl Ester

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, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 7.6 at 25°C with 1 M NaOH.)

B. 0.25 mM N□-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 1 mg protein/ml of Trypsin, Prod. No. T-8003, in cold Reagent C.)

E. Trypsin Inhibitor Solution (Inhib)
(Immediately before use, prepare a solution containing 1.0 mg/ml of Trypsin Inhibitor in cold Reagent A.[2,3])

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PROCEDURE:

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

Part A:

	Uninh	Test1	Test2	Test3	Test4	Test5
Reagent E (Inhib)	---	0.10	0.15	0.20	0.25	0.30
Reagent D (Trypsin)	0.50	0.50	0.50	0.50	0.50	0.50
Reagent C (HCl)	9.50	9.40	9.35	9.30	9.25	9.20

Allow to stand at 25°C for a minimum of five minutes and no longer than six minutes.

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

Part B:

	Uninh	Test1	Test2	Test3	Test4	Test5	Blank
Reagent B (BAEE)	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Reagent C (HCl)	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 ΔA_{253nm}/minute using the maximum linear rate for the Tests, Blank, and Uninhibited Solution.

CALCULATIONS:

Trypsin Activity in BAEE units/ml enzyme =

$$\frac{(\Delta A_{253nm}/\text{min Test} - \Delta A_{253nm}/\text{min Blank})(df)(10.0)}{(0.001)(0.10)(0.5)}$$

df = Dilution factor
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
0.10 = Volume (in milliliters) enzyme used (Part B)
10.0 = Total volume in milliliters of assay (Part A)
0.5 = Volume (in milliliters) of enzyme used (Part A)

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CALCULATIONS: (continued)

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

Plot the Trypsin activity (BAEE units/mg protein) vs ml of Trypsin Inhibitor/RM

Mg Trypsin Inhibitor = (ml of Trypsin Inhibitor)(Conc. of Trypsin Inhibitor, mg/ml)

$$\text{Mg Trypsin Inhibited by 1 mg Trypsin Inhibitor} = \frac{\text{mg Trypsin/RM (normalizing factor)}}{\text{mg Trypsin Inhibitor (from plot)}}$$

Normalizing Factor = (BAEE Units of Uninhibited Trypsin per mg solid/10,000 BAEE units of Trypsin per specification.)

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.

FINAL ASSAY CONCENTRATION:

In a 3.20 ml reaction mix, the final concentrations are 63 mM sodium phosphate, 0.23 mM BAEE, 0.002 mM HCl, 0.005 mg trypsin, and 0.0003 - 0.001 mg trypsin inhibitor.

NOTES:

1. This enzyme assay is used to assay Sigma Prod. Nos. T-9003, T-9008, T-9128, T-9253, T-2011, T-1886, T-4385, T-9378, and T-0256.

2. When assaying Trypsin Inhibitor, Ovoinhibitor, Prod. No. T-1886, the diluent used is 200 mM sodium phosphate, monobasic, pH 7.6 at 25°C.

3. When assaying Trypsin Inhibitor, Type II-S, Sigma Prod. No. T-9128, prepare a solution containing 0.60 mg/ml of Trypsin Inhibitor in cold Reagent A.

4. The uninhibited Trypsin activity should be within 85% of the release value for activity. With a 11,700 to 13,005 Trypsin units/mg solid per label, the acceptable range for activity of the uninhibited Trypsin reaction should be 10,000 to 15,300 Trypsin units/mg solid. This range should also correspond to a corrected ΔAbs_{253nm}/minute of 0.0545 to 0.0835. With this rate and an inhibition of 20% to 80% the ΔAbs_{253nm}/minute should be above the spectophotometric rate detection limit of 0.0020.

NOTES: (continued)

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.