Creative Enzymes

Enzymatic Assay of TRYPSIN TPCK/DITC GLASS (EC 3.4.21.4)

PRINCIPLE:

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

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

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

METHOD: Spectrophotometric Rate Determination

REAGENTS:

A. 400 mM Sodium Phosphate Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous.
Adjust to pH 7.6 with 1 M HCl.)

B. 0.26 mM Na-Benzoyl-L-Arginine Ethyl Ester Solution (BAEE)
(Prepare 50 ml in Reagent A using Na-Benzoyl-L-Arginine Ethyl Ester, Hydrochloride.)

C. Trypsin Enzyme Standard Solution (STD)
(Immediately before use, prepare a solution containing 0.01 mg/ml of Trypsin, in cold Reagent A.)

D. Trypsin Insoluble Enzyme (Trypsin/DITC)
(Use Trypsin, TPCK Bound to DITC glass.)

PROCEDURE:

Step 1: Standard Determination

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

	Standard	Blank
Reagent B (BAEE)	3.10	3.10

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PROCEDURE: (continued)

Equilibrate to 25°C. Then add:

	Standard	Blank
Reagent C (Std)	0.10	------
Reagent A (Buffer)	------	0.10

Immediately mix by inversion and incubate at 25°C for exactly 1.5 minutes. Record the A_{253nm} for the Standard and Blank using a suitably thermostatted spectrophotometer.

Step 2: Sample Determination

Weigh (in milligrams) the following reagent into a suitable container.

	Test	Blank
Reagent D (Trypsin/DITC)	5-10	------

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

Reagent A (Buffer)	0.10	0.10
Reagent B (BAEE)	3.10	3.10

Immediately vortex at a medium speed for exactly 1.5 minutes. Then filter the Test and Blank through a 0.45 µm syringe filter. Record the A_{253nm} for both the Test and Blank filtrate using a suitable spectrophotometer.

CALCULATION:

Standard:

$$\text{Units/ml enzyme} = \frac{(A_{253nm}\ \text{Test} - A_{253nm}\ \text{Blank})(df)}{(0.001)(1.5)}$$

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 mixture
1.5 = Time (in minutes) of assay

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CALCULATION: (continued)

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

Compare the activity of the standard to its release value (activity). This is to ensure that the assay is functioning correctly.

Test:

$$\text{Units/g enzyme} = \frac{(A_{253nm}\ \text{Test} - A_{253nm}\ \text{Blank})(1000)}{(0.001)(1.5)(\text{mg Trypsin bound to DITC glass})}$$

1000 = Conversion from mg to g
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
1.5 = Time (in minutes) of assay

UNIT DEFINITION:

One BAEE unit will produce a A_{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 3.2 ml reaction mix, the final concentrations are 400 mM sodium phosphate, 0.25 mM Na-benzoyl-L-arginine ethyl ester, 5 - 10 mg of trypsin, TPCK bound to DITC glass.

REFERENCE:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Volume I, 2nd ed., 515-516, Academic Press Inc., New York NY

NOTES:

1. This assay is based on the cited reference.

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This procedure is for informational purposes.

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