

Enzymatic Assay of α -CHYMOTRYPSIN (Insoluble)
(EC 3.4.21.1)

PRINCIPLE:

ATEE + H₂O $\xrightarrow{\alpha\text{-Chymotrypsin}}$ N-Acetyl-L-Tyrosine + Ethanol

Abbreviation used:

ATEE = N-Acetyl-L-Tyrosine Ethyl Ester

CONDITIONS: T = 30°C, pH 8.0

METHOD: Titrimetric

REAGENTS:

- A. 50% (w/w) Methanol Solution (MeOH)
(Prepare 25 ml in deionized water using Methanol, Absolute, Sigma Stock No. 17-5.)
- B. 50 mM N-Acetyl-L-Tyrosine Ethyl Ester (ATEE)
(Prepare 20 ml in Reagent A using N-Acetyl-L-Tyrosine Ethyl Ester, Sigma Prod. No. A-6751.)
- C. 500 mM Calcium Chloride Solution (CaCl₂)
(Prepare 25 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)
- D. 100 mM Sodium Hydroxide Solution Standardized (NaOH)
(Prepare 100 ml in deionized water using Sodium Hydroxide, Anhydrous, Sigma Stock No. 505-8. Standardize according to the ACS Reagent Procedure.¹)
- E. α -Chymotrypsin Enzyme, Insoluble (Enzyme)
(Use α -Chymotrypsin, Insoluble, 5 - 10 mg.)

PROCEDURE:

Weigh (in milligrams) the following reagents into a suitable container:

	<u>Test</u>
Reagent E (Enzyme)	5.00 - 10.00

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PROCEDURE: (continued)

Then pipette (in milliliters) the following reagents:

	<u>Test</u>
Deionized Water	6.00
Reagent C (CaCl ₂)	2.00

Mix by swirling and adjust the pH to 8.4 with Reagent D.
Equilibrate to 30°C and then add:

Reagent B (ATEE)	2.00
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Mix by swirling and incubate at 30°C. When the pH reaches 8.0 begin timing the reaction. Run the reaction for 1 - 5 minutes. Maintain the pH of the reaction mix at 8.0 by the addition of small volumes (0.10 ml) of Reagent D. Record the volume of Reagent D used to maintain the pH at 8.0 and the time required.

CALCULATIONS:

$$\text{Units/mg solid} = \frac{(\text{Molarity of NaOH})(\text{NaOH})(1000)}{(\text{mg solid})(T)}$$

NaOH = Volume (in milliliters) of Reagent D used in the assay

1000 = Conversion factor to μ moles from millimoles
mg solid = mg solid of α -chymotrypsin, insoluble, used in the assay

T = Time of assay (in minutes) required to maintain the pH at 8.0

UNIT DEFINITION:

One unit will hydrolyze 1.0 μ mole of ATEE per minute at pH 8.0 at 30°C.

INITIAL ASSAY CONCENTRATIONS:

In a 10.00 ml reaction mix, the initial concentrations are 100 mM calcium chloride, 10 mM N-acetyl-L-tyrosine ethyl ester, and 5 - 10 mg of α -chymotrypsin, insoluble.

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REFERENCES:

- Laskowski, M. (1955) *Methods in Enzymology*, Volume II, 2-26.
- (1993) *Reagent Chemicals ACS Specification*, 8th ed., p. 95, American Chemical Society, Washington, D.C.

NOTES:

1. Standardization of the NaOH solution is described in (1993) *Reagent Chemicals ACS Specification*.
2. This assay is based on the cited references.
3. 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.