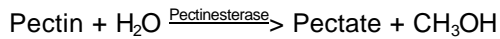


Enzymatic Assay of PECTINESTERASE (EC 3.1.1.11)

PRINCIPLE:



CONDITIONS: T = 30°C, pH = 7.5

METHOD: Titrimetric

- A. 100 mM Sodium Chloride Solution (NaCl)
(Prepare 300 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-7653.)
- B. 1.0% (w/v) Pectin Solution (Pectin)
(Prepare 250 ml in Reagent A using Pectin Prod. No. P-9135. Dissolve the Pectin into approximately 150 ml of Reagent A which has been heated to boiling and stir with a magnetic stirrer. Cool to room temperature upon solubilizing and dilute to 250 ml with Reagent A.¹⁾)
- C. 20 mM Sodium Hydroxide Solution - Standardized (NaOH)
(Prepare 50 ml in deionized water using Sodium Hydroxide, Anhydrous, Sigma Stock No. 505-8. Standardize according to the ACS Reagent Procedure.²⁾)
- D. 1.7 M Sodium Chloride Solution (Enzyme Diluent)
(Prepare 50 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-7653.)
- E. Pectinesterase Enzyme Solution
(Immediately before use, prepare a solution containing 10 - 15 units/ml of Pectinesterase in cold Reagent D.)

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PROCEDURE:

Using a suitable pH meter in conjunction with a magnetic stirrer, pipette (in milliliters) the following reagents into a suitably thermostatted titration vessel:

	<u>Test</u>	<u>Blank</u>
Reagent B (Pectin)	20.00	20.00
Equilibrate to 30°C. Adjust to pH 7.5 with Reagent C (NaOH). Then add:		
Reagent E (Enzyme Solution)	0.20	-----
Reagent D (Enzyme Diluent)	-----	0.20

Run the reaction for 1-5 minutes. Record the time when the pH reaches 7.5. Maintain the pH of the reaction mix at pH 7.5 by the addition of small volumes (0.05 ml) of Reagent C (NaOH). Record the volume of Reagent C (NaOH) used to maintain the pH at 7.5 and the time required.

CALCULATION:

$$\text{Units/ml enzyme} = \frac{(M) \left(\frac{\text{ml NaOH for Test}}{T} - \frac{\text{ml NaOH for Blank}}{T} \right) (1000) (df)}{(T)(0.20)}$$

M = Molarity of NaOH solution
 1000 = Conversion from milliequivalent to microequivalents
 df = Dilution factor
 T = Time required to maintain the pH at 7.5
 0.2 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will release 1.0 microequivalent of acid from pectin per minute at pH 7.5 at 30EC.

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INITIAL ASSAY CONCENTRATIONS:

In a 21.00 ml reaction mix, the initial concentrations are 176 mM sodium chloride, 0.95% (w/v) pectin, and 2 - 3 units pectinesterase.

REFERENCES:

(1993) *Reagent Chemical ACS Specification*, 8th ed., 95.

Kertesz, Z.I. (1955) *Methods in Enzymology*, Vol. I, 158-162.

NOTES:

1. The solution must be agitated during preparation to prevent pectin from adhering to the bottom of container and being burned.
2. Standardization of NaOH solution is described in (1993) *Reagent Chemical ACS Specification*, 8th ed., 95.
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.