Enzymatic Assay of PECTIN LYASE
(EC 4.2.2.10)

PRINCIPLE:

$$\text{Pectin} + H_2O \xrightarrow{\text{Pectin Lyase}} \text{4-Deoxy-6-Methyl-?-4,5-Galacturonic Acid Oligomers}$$

CONDITIONS: T = 40°C, pH = 6.0, A_{235nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Citrate and 100 mM Phosphate Buffer, pH 6.0 at 40°C
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Anhydrous, Sigma Prod. No. C-0759, and Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 6.0 at 40°C with 1 M NaOH.)

B. 0.5% (w/v) Pectin Solution (Pectin)
(Prepare 50 ml in Reagent A using Pectin, from Citrus Fruits, Sigma Prod. No. P-9135. Mild heating for 4 - 5 minutes, with stirring, is required to dissolve the pectin. Filter through a 0.22 µm filter to clarify.)

C. 100 mM Citrate and 100 mM Phosphate Buffer with 0.1% (w/v) Bovine Serum Albumin (Enzyme Diluent)
(Prepare 25 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503.)

D. Pectin Lyase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 0.5 unit/ml in cold Reagent C.)

PROCEDURE:

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

	Test	Blank
Reagent B (Pectin)	1.80	1.80
Reagent A (Buffer)	0.20	0.20

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 40°C. Monitor the A_{235nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.50	------
Reagent C (Enzyme Diluent)	------	0.50

Immediately mix by inversion and record the increase in A_{235nm} for approximately 5 minutes. Obtain the ΔA_{235nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{235nm}/\text{min Test} - \Delta A_{235nm}/\text{min Blank})(2.5)(df)}{(1.0)(0.5)}$$

2.5 = Total volume (in milliliters) of assay
df = Dilution factor
1.0 = Change in A_{235nm} per minute at 40°C as per the Unit Definition
0.5 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will cause a ΔA_{235} of 1.0 per minute at 40°C due to the release of unsaturated products from Pectin (P-9135).

FINAL ASSAY CONCENTRATION:

In a 2.50 ml reaction mixture, the final concentrations are
100 mM citric acid, 100 mM sodium phosphate,
0.4% (w/v) pectin, 0.02% (w/v) bovine serum albumin and
0.15 - 0.25 unit pectin lyase.

REFERENCE:

Albersheim, P. (1966) *Methods in Enzymology*, Vol. 8, 628-631

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NOTES:

1. This assay is based on the cited reference.

2. 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.