Enzymatic Assay of PECTIN LYASE  
(EC 4.2.2.10)

PRINCIPLE:

Pectin + H₂O > 4-Deoxy-6-Methyl-α,4,5-Galacturonic Acid Oligomers

CONDITIONS:  T = 40°C, pH = 6.0, A₄₃₅nm, 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Pectin)</td>
<td>1.80</td>
<td>1.80</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

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

<table>
<thead>
<tr>
<th>Reagent D (Enzyme Solution)</th>
<th>0.50</th>
<th>------</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Enzyme Diluent)</td>
<td>------</td>
<td>0.50</td>
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</tbody>
</table>

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

CALCULATIONS:

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

2.5 = Total volume (in milliliters) of assay  
df = Dilution factor  
1.0 = Change in $A_{235\text{nm}}$ 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 $r_{A_{235}}$ 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:

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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.