



PECTATE LYASE from *Aspergillus sp.* (Lot 10801)

E-PECLY1

09/03

PROPERTIES

1. ELECTROPHORETIC PURITY

- Single major band on SDS-gel electrophoresis (MW = 32,000).

2. SPECIFIC ACTIVITY AND LEVEL OF OTHER ACTIVITIES

SUBSTRATE	SPECIFIC ACTIVITY (U/mg Protein)
Polygalacturonic acid (pectate lyase)	19.5 (pH 10.8)(CAPS buffer)*
Polygalacturonic acid (pectate lyase)	5.0 (pH 8.0)(Tris-Bispropane)
Polygalacturonic acid (pectate lyase)	2.2 (pH 8.0) (Tris HCl buffer)*
Polygalacturonic acid (<i>endo</i> -Polygalacturonanase)	0.000001
Galactzyme Tablets (<i>endo</i> -Galactanase)	0.0000003
Arabinzyme Tablets (<i>endo</i> -Arabinanase)	0.00001

* The activity of this enzyme was previously stated to be 89 Units per mg of protein. This value was incorrect due to a calculations error. The correct values are as shown in the table.

3. Enzyme activity

Pectate lyase was assayed at pH 8.0 in Tris-HCl buffer or in Tris-Bispropane buffer and at pH 10.8 in CAPS buffer, and at 40°C with polygalacturonic acid as substrate. Activity was monitored at 235 nm in a recording spectrophotometer. *endo*-Polygalacturonanase, *endo*-arabinanase and *endo*-galactanase were assayed at pH 4.5 and 40°C.

3. PHYSICOCHEMICAL PROPERTIES

pH optima 10.8.

pH stability 6.5-11.0.

Temperature optima 50°C.

Temperature stability < 50°C.

A solution of enzyme at 1 mg protein/ml (Folin lowry) has an abs. of 1.35 at 280 nm.

4. STORAGE CONDITIONS

The enzyme is supplied as a solution of approximately 120 Units/ml (at pH 10.8) in 50% glycerol plus 0.02% sodium azide.

Store at 4°C (or at -20°C long term).

5. ASSAY OF PECTATE LYASE ACTIVITY

Pectate lyase (Megazyme preparation, ~ 120 U/mL) is diluted in 50 mM CAPS buffer (pH 10.8) containing 1 mM CaCl₂ to a final enzyme concentration of approximately 0.07 U/mL (i.e. a dilution of 200-fold).

Polygalacturonic acid (Megazyme cat. no. P-PGACT) is dissolved to a concentration of 2.5 mg/ml in 50 mM CAPS buffer (pH 10.8) containing 1 mM CaCl₂.

Assay Procedure:

To a 1 cm lightpath quartz cuvette in the heating chamber of the recording spectrophotometer,

Add: 1.0 mL of polygalacturonic acid (2.5 mg/ml) solution in CAPS buffer, plus
1.0 mL of CAPS buffer (50 mM, pH 10.8) plus 1 mM CaCl₂.

allow to equilibrate to 40°C over 5 min.

Then add:

0.5 mL of suitably diluted enzyme solution.

Mix the solution well and measure the absorbance increase at 235 nm over a period of 20 min.

Prepare enzyme and substrate blanks, by replacing these components with an equal volume of Tris/HCl buffer, and run the reactions concurrently with the enzyme-substrate reaction.

Measure the initial rate of reaction in the linear part of the kinetic reaction curve.

CALCULATIONS:

Activity, Units/mL of original solution:

$$= A / T \times 1/4.6 \times 2.5/0.5 \times \text{Dilution.}$$

where:

A/ T	= rate of increase in absorbance at 235 nm.
4.6	= absorption coefficient of the unsaturated bond at the 4-5 position of the uronic acid residue (i.e. $_{235} = 4.6 \text{ mmol}^{-1} \times \text{cm}^{-1}$).
2.5	= the total volume of the reaction mixture.
0.5	= the volume of enzyme used in the assay.
Dilution	= dilution of the original enzyme preparation (e.g. for Megazyme Pectate Lyase Lot 10801 = 2000).

Thus:

$$\begin{aligned} &\text{Activity, Units/mL of original Megazyme preparation (Lot 10801)} \\ &= 0.55/10 \times 1/4.6 \times 2.5/0.5 \times 2000 \\ &= 120 \text{ Units/mL.} \end{aligned}$$

Assay for the Identification of Pectin

(Based on Hansen, K.M., Thuesen, A. B. and Soderberg, J. R. (2001) "Enzyme assay for identification of pectin and pectin derivatives, based on recombinant pectate lyase". *J. AOAC International*, **84**, 1851).

Chemicals:

Tris (Hydroxymethyl) aminomethane. Trizma Base (Sigma Chemical Co. cat. no. T-8524)
Calcium chloride-dihydrate (Merck).

Buffers and Reagents:

- Tris/HCl buffer plus CaCl_2 .- Dissolve 6.055 g of Trizma base and 0.147 g of calcium chloride dihydrate in 900 mL of deionised water. Adjust pH to 8.0 with 1 M HCl. Adjust volume to 1 litre. Store at 4°C.
- 0.5 M NaOH.- Dissolve 20 g of NaOH in 1 litre of deionised water.
- 0.5 M HCl.- Add 50 mL of conc. HCl (10 M) to 950 mL of deionised water.
- 1 M HCl.- Add 100 mL of conc HCl (10 M) to 900 mL deionised water.
- 2-Propanol (100%).

Pectate Lyase (from Megazyme, cat no. E-PECLY):

The enzyme used in this work was purified from a crude *Aspergillus* sp. recombinant preparation. The purified pectate lyase gives a single band on SDS-gel electrophoresis and has a pH optima of 10.8, but is used at pH 8.0 in this assay. It is supplied at approximately 14 Units/mL (at pH 8.0 in Tris/HCl buffer) in a solution of 50% glycerol. The enzyme, as supplied, is stable at 4°C for at least 2 years, and for greater than 5 years at -20°C.

For use in this assay, 0.5 mL of enzyme is diluted to 50 mL with Tris/HCl buffer (pH 8.0). (i.e. a 100-fold dilution). The enzyme in buffer is stored in suitable aliquots in polypropylene containers at -20°C between use, and is stable to multiple freezing and thawing cycles.

Sample Preparation:

- Moisten 50 milligrams (0.05 g) of the sample with 0.1 ml of 2-propanol.
- Add 50 mL of deionised water and stir gently on a magnetic stirrer for 2 hours.
- Adjust the pH to 12 by careful addition of 0.5 M NaOH, and leave the solution for exactly 15 min at room temperature.
- Lower the pH to 8.0 by dropwise addition of 0.5 M HCl.
- Adjust the volume to 100 mL with deionised water.

Measurement of the samples:

Add the following to quartz cuvettes:

	Tris-HCl buffer (pH 8)	Sample	Deionised water	Diluted enzyme
Enzyme blank	0.5 mL	1.0 mL	1.0 mL	-
Sample blank	0.5 mL	-	1.5 mL	0.5 mL
Reaction	0.5 mL	1.0 mL	0.5 mL	0.5 mL

The contents of the cuvettes are mixed well, and the absorbance values are measured at 235 nm after 30 min.

Results:

The increase in absorbance for a given sample on incubation with pectate lyase is measured as follows:

Blank Absorbance = Enzyme Blank + Sample Blank (measured after 30 min).

Absorbance = **Reaction Absorbance** – **Blank Absorbance**.

From the increase in absorbance (**Abs.**), the amount of unsaturated product produced can be calculated as:

Unsaturated product = $\frac{\text{Abs./l}}{\epsilon} \times \dots$

where:

Abs. = Reaction Absorbance (after 30 min) – Blank Absorbance

l = path length of the reaction cuvette (= 1 cm).

ϵ = the molar extinction coefficient of the reaction product ($4600 \text{ M}^{-1} \text{ cm}^{-1}$).

This assay can be performed in a standard UV spectrophotometer simply by measuring the absorbance values of the reaction solution and the two blank solutions after the 30 min incubation. However, the reaction can also be followed in a recording UV spectrophotometer set-up for kinetic assays.

The results for such a study is shown in Figure 1.

Incubation conditions:

1.0 ml of sample (0.5 mg/mL) + 0.5 mL deionised water + 0.5 mL Tris/HCl buffer (containing CaCl_2)

Add: 0.5 mL of pectate lyase (Megazyme product diluted 1:100 in Tris/HCl buffer; i.e. 0.14 U/mL)

Mix immediately and follow absorbance increase at 235 nm in a recording spectrophotometer thermostatted at 40°C .

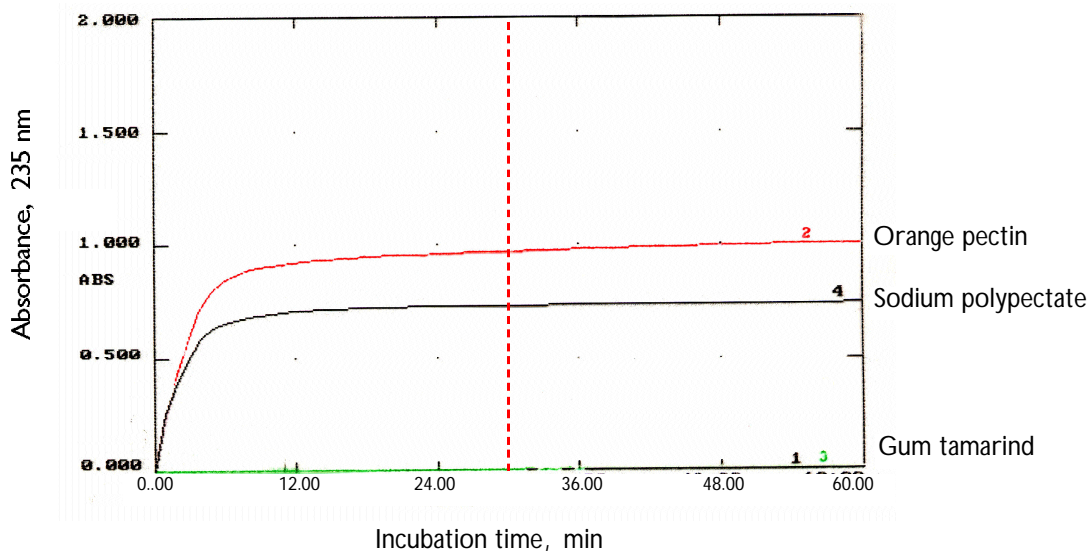


Figure 1. Increase in absorbance at 235 nm on incubation of various polysaccharides with pectate lyase.

The results obtained for a number of pectin samples (as supplied by Annette B.Thusen, CP Kelco ApS) are shown below:

Sample number	Manufacturer	Polysaccharide type	Absorbance Values				Unsaturated Product x 10 ⁻⁴
			Sample	Enzyme blank	Reaction blank	Abs.	
101	Danisco	Lime pectin	0.080	0.094	0.689	0.515	1.12
102	Danisco	Orange pectin	0.080	0.065	0.713	0.568	1.23
103	Citrus colloids	Unstandardised low methoxyl pectin	0.080	0.161	1.034	0.793	1.72
104	Citrus Colloids	Sodium polypectate	0.080	0.055	0.742	0.607	1.32
105	Herbstreith & Fox	HM apple pectin	0.080	0.113	0.642	0.449	0.98
106	Citrus Colloids	HM citrus pectin (all process)	0.080	0.046	0.807	0.681	1.48
107	Copenhagen pectin	Gum tamarind	0.080	0.045	0.100	(-0.025)	0.00
108	Obipectin	Type OP (Lot 9714)	0.080	0.099	0.836	0.657	1.43
109	Obipectin	Type D-OP (Lot 9719)	0.080	0.027	0.551	0.444	0.96
110	Copenhagen pectin	Beta-52-4346-57	0.080	0.110	0.764	0.574	1.25
111	SBUI	LM amide apple pectin Nr. Envoi 12993	0.080	0.111	0.576	0.385	0.84
112	Copenhagen pectin	C52 (carrageenan) 45-50720-21	0.080	0.112	0.066	(-0.126)	0.00

Calculations:

$$\begin{aligned} \text{Unsaturated Product} &= \text{Absorbance (30 min)} \times 1/ \times 1/L \\ &= \text{Absorbance (30 min)} \times 1/4600 \times 1.0 \end{aligned}$$

where:

Absorbance = **Reaction Absorbance** (= Absorbance after 30 min – **Blank Absorbance**).

= molar extinction coefficient (= 4600 M⁻¹ cm⁻¹).

L = cuvette path length (= 1 cm).