

Megazyme

PECTIN IDENTIFICATION

ASSAY PROCEDURE

K-PECID 11/11

(500 Assays per Kit)



INTRODUCTION:

Pectins consist of the partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium and ammonium salts, obtained by extraction in an aqueous medium of appropriate edible plant material, usually citrus fruits or apples. Pectin is recovered from solution by precipitation with an appropriate organic solvent such as ethanol, methanol or isopropanol. In some pectins, a portion of the methyl esters may have been converted to primary amide groups by treatment with ammonia under alkaline conditions. Older methods for the identification of pectin involve solvent precipitation. These methods are non-specific. The method described here measures the increase in absorption at 235 nm on cleavage of pectate by a highly purified and specific enzyme, pectate lyase¹. The enzyme employed since 2011 is still derived from *Aspergillus Niger*, but it is different from that employed up until this date (see Figure 2).

PRINCIPLE:

Pectin is dissolved in deionised water and adjusted to pH 12.0 to catalyse demethylation with the production of polygalacturonic acid regions in the polymer i.e. conversion of pectin to pectate. The pectate is incubated with pectate lyase which cleaves the polygalacturonic acid, releasing unsaturated oligosaccharides which absorb strongly at 235 nm. The JECFA method² for the identification of pectins is very similar to the current method, however, the pectate lyase supplied with this kit acts better at pH 8.0.

KITS:

Kits suitable for performing 500 assays are available from Megazyme and consist of:

1. Concentrated Pectate Lyase enzyme
2. Low ester pectin extracted from citrus peel
3. High ester pectin extracted from citrus peel
4. Partly amidated low ester pectin from citrus peel
5. Pectin from sugar beet pulp
6. Iota carrageenan

REFERENCES:

1. 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**, 1851-1854.
2. Compendium of Food Additive Specifications, FAO Food and Nutrition paper 52, Add. 9, Joint FAO/WHO Expert Committee on Food Additives (JECFA) 57th session, Rome, Italy, 5-14th June, 2001.

SPECIFICITY:

The assay is absolutely specific for polygalacturonic acid.

ENCLOSED ENZYME:

Pectate Lyase (2.7 mL, 1400 U/mL at pH 8.0; Tris buffer)
(500 U/mL at pH 10.8; CAPS buffer).

NOTE: As of December 2011, a new form of pectate lyase is being used in this kit. This enzyme is purified from an *Aspergillus* sp. recombinant preparation. The purified pectate lyase appears as a single major band on SDS-gel electrophoresis and has a pH optima of 8. It is supplied in 50% glycerol plus preservative. Stable for > 5 years at -20°C.

For use in the assay of pectin, dilute 0.5 mL of enzyme to 50 mL in Tris-HCl buffer (pH 8.0) (i.e. a 100-fold dilution). Once dissolved, the enzyme is stable for > 2 years at -20°C and to multiple freezing and thawing cycles.

ENCLOSED STANDARDS:

1. Low ester pectin extracted from citrus peel
2. High ester pectin extracted from citrus peel
3. Partly amidated low ester pectin from citrus peel
4. Pectin from sugar beet pulp
5. Iota carrageenan

BUFFERS AND REAGENTS:

1. **50 mM Tris/HCl buffer plus 1 mM CaCl₂.**
Dissolve 6.055 g of Trizma base (Sigma Cat. no. T-1503) 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. Stable at 4°C for approx. 2 weeks.
2. **0.5 M NaOH.**
Dissolve 20 g of NaOH in 1 litre of deionised water.
Stable at room temperature for > 2 months.
3. **0.5 M HCl.**
Add 50 mL of conc. HCl (10 M) to 950 mL of deionised water.
Stable at room temperature for > 2 months.
4. **1 M HCl.**
Add 100 mL of conc. HCl (10 M) to 900 mL deionised water.
Stable at room temperature for > 2 months.
5. **2-Propanol (100%).**

SAMPLE PREPARATION:

1. Moisten 50 mg (0.05 g) of the sample with 2 drops of 2-propanol.
2. Add 50 mL of deionised water and stir gently on a magnetic stirrer for 20-30 min (until the pectin dissolves).
3. Adjust the pH to 12 by careful addition of 0.5 M NaOH, and leave the solution for exactly 15 min at room temperature.
4. Lower the pH to 8.0 by dropwise addition of 0.5 M HCl.
5. Adjust the volume to 100 mL with deionised water.

Add the following to quartz cuvettes, mix the contents well, and measure the absorbance values at 235 nm after 30 min.

	Tris-HCl buff. (pH 8.0)	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

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).

Δ Absorbance = Reaction Absorbance – Blank Absorbance.

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

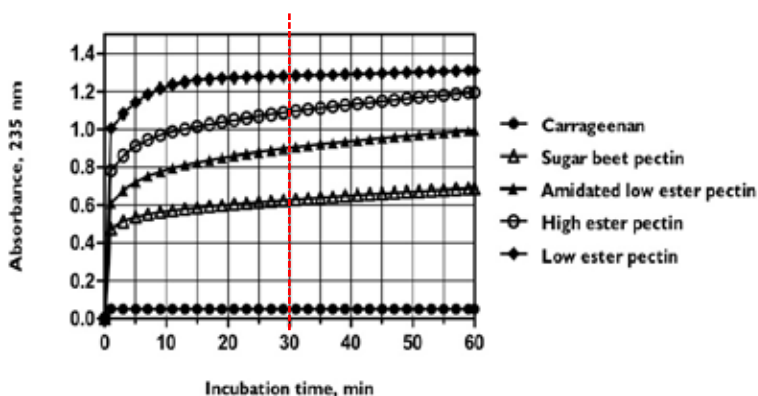
Unsaturated product = $\Delta A/L \times \epsilon$

where:

- ΔA = 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 M⁻¹ 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 are shown in Figure 1.

Figure 1. Increase in absorbance at 235 nm on incubation of pectic polysaccharides (or carrageenan) with pectate lyase



Incubation conditions:

1.0 mL of sample (0.5 mg/mL) + 0.5 mL deionised water + 0.5 mL Tris-HCl buffer (pH 8.0, containing 1 mM CaCl_2).

Add: 0.5 mL of diluted pectate lyase (14 U/mL).

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

Table 1. Determination of the content of unsaturated oligosaccharides in pectic and non-pectic polysaccharides

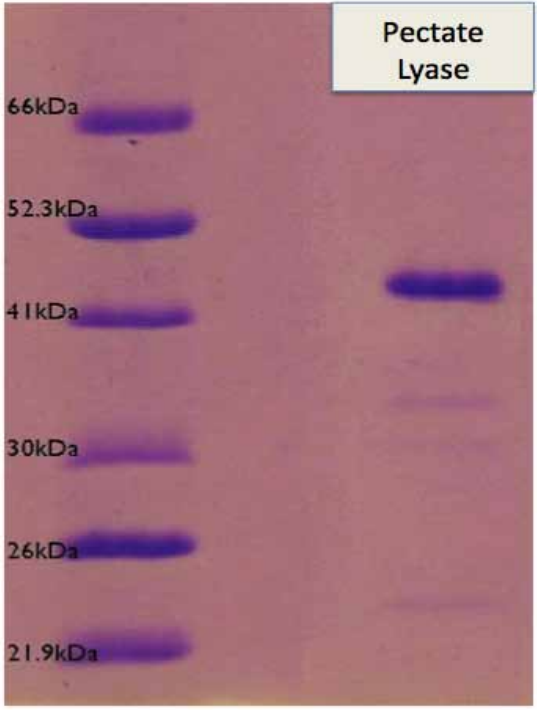
Polysaccharide type	Absorbance values			Unsaturated product x 10 ⁻⁴
	Enzyme Blank	Sample Blank	Sample Reaction	
Amidated low ester pectin (lot 20501)	0.005	0.049	0.910	1.86
Sugar beet pectin (lot 20502)	0.005	0.049	0.593	1.17
Carragenan (lot 20503)	0.003	0.047	0.052	0.004
High ester pectin (lot 20504)	0.004	0.047	1.117	2.32
Low ester pectin (lot 20505)	0.001	0.050	1.263	2.63

Calculations:

$$\text{Unsaturated product} = \Delta A (30 \text{ min}) \times l/\epsilon \times 1/L$$

where: ΔA = Sample Reaction Absorbance - Enzyme Blank - Sample Blank
 L = path length (= 1 cm)
 ϵ = molar extinction coefficient of the reaction product (4600 M⁻¹cm⁻¹)

Figure 2. SDS gel electrophoresis of pectate lyase and molecular weight standards.



NOTES:



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