



Pectate Lyase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1093

(Version 1.3D)

Detection and Quantification of Pectate Lyase (PL) Activity in Tissue extracts, Cell lysate and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Pectate lyase (EC 4.2.2.2) is an enzyme involved in the maceration and soft rotting of plant tissue. Pectate lyase is responsible for the eliminative cleavage of pectate, yielding oligosaccharides with 4-deoxy- α -D-mann-4-enuronosyl groups at their non-reducing ends. The protein is maximally expressed late in pollen development. It has been suggested that the pollen expression of pectate lyase genes might relate to a requirement for pectin degradation during pollen tube growth.

This enzyme catalyzes the chemical reaction

Eliminative cleavage of (1 $\rightarrow$ 4)- α -D-galacturonan to give oligosaccharides with 4-deoxy- α -D-galact-4-enuronosyl groups at their non-reducing ends.

Pectate Lyase Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring pectate lyase activity in a variety of samples. The enzyme catalysed reaction products Oligogalacturonic acid, can be measured at a colorimetric readout at 235 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well UV Microplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Substrate	18 mlx 1	4 °C
Stop Solution	20mlx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 235 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 10,000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent*	Sample**	Control
Sample	10 μ l	--
Boiled Sample	--	10 μ l
Substrate	90 μ l	90 μ l
Mix, put it in the oven, 50°C for 15 minutes.		
Stop Solution	100 μ l	100 μ l
Centrifuged at 6,000g for 5 minutes, add 100 μ l supernatant into the microplate, record absorbance measured at 235 nm.		

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VI. CALCULATION

Unit Definition: One unit of Pectate lyase activity is defined as the enzyme generates 1 μmol of Oligogalacturonic acid per minute.

1.1 According to the protein concentration of sample

$$\text{Activity} = \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\epsilon \times d \times C_{\text{Protein}} \times T} \times \frac{V_{\text{Total}}}{V_{\text{Sample}}} (\text{U/mg})$$

1.2 According to the weight of sample

$$\text{Activity} = \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\epsilon \times d \times W \times T} \times \frac{V_{\text{Total}} \times V_{\text{Assay}}}{V_{\text{Sample}}} (\text{U/g})$$

1.3 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\epsilon \times d \times N \times T} \times \frac{V_{\text{Total}} \times V_{\text{Assay}}}{V_{\text{Sample}}} (\text{U}/10^4)$$

1.4 According to the volume of sample

$$\text{Activity} = \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\epsilon \times d \times T} \times \frac{V_{\text{Total}}}{V_{\text{Sample}}} (\text{U/mg})$$

ε: molar extinction coefficient, $5.2 \times 10^3 \text{ L/mol/cm} = 5.2 \text{ ml}/\mu\text{mol/cm}$

d: the optical path of 96-Well microplate, 0.3 cm

C_{Protein} : the protein concentration, mg/ml

W: the weight of sample, g

N: the quantity of cell or bacteria, $N \times 10^4$

V_{Total} : the total volume of all reagents in assay procedure, mL

V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minutes