Pectate Lyase Microplate Assay Kit

User Manual

Catalog # CAK1093

(Version 1.2D)

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→4)-α-D-galacturonan to give oligosaccharides with 4-deoxy-α-D-galact-4-enuronosyl groups at their non-reducing ends.

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	30 ml x 4	4 °C
Substrate	18 ml x 1	4 °C
Stop Solution	20 ml x 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 µl	--
Boiled Sample	--	10 µl
Substrate	90 µl	90 µl
Mix, put it in the oven, 50 °C for 15 minutes.		
Stop Solution	100 µl	100 µl
Centrifuged at 5,000g for 5 minutes, add 100 µl supernatant into the microplate, record absorbance measured at 235 nm.		

Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

$$PL\ (U/mg) = (OD_{Sample} - OD_{Control}) / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.855 \times (OD_{Sample} - OD_{Control}) / C_{Protein}$$

2. According to the weight of sample

$$PL\ (U/g) = (OD_{Sample} - OD_{Control}) / (\varepsilon \times d) \times V_{Total} / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 0.855 \times (OD_{Sample} - OD_{Control}) / W$$

3. According to the quantity of cells or bacteria

$$PL\ (U/10^4) = (OD_{Sample} - OD_{Control}) / (\varepsilon \times d) \times V_{Total} / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 0.855 \times (OD_{Sample} - OD_{Control}) / N$$

4. According to the volume of sample

$$PL\ (U/ml) = (OD_{Sample} - OD_{Control}) / (\varepsilon \times d) \times V_{Total} / V_{Sample} / T$$

$$= 0.855 \times (OD_{Sample} - OD_{Control})$$

ε: molar extinction coefficient, 5.2×10^3 L/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 the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 15 minutes.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES