Pectate Lyase Assay Kit

Pectate Lyase Assay Kit is a detection kit for the quantification of Pectate Lyase Activity in tissue extracts and cell lysate.

Catalog number: ARG82026

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 621 8100

Fax: +886 (3) 553 0266

Email: info@arigobio.com

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. [Provide by Wikipedia: Pectate lyase]

PRINCIPLE OF THE ASSAY

This Pectate Lyase Assay Kit is a simple assay that measures the amount of pectate lyase activity in tissue extracts and cell lysate. The assay is based on the enzyme driven reaction. 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 O.D. 235 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well UV Microplate	1 plate	RT
Assay Buffer	4 x 30 mL (ready to use)	4°C
Substrate	18 mL (ready to use)	4°C
Stop Solution	20 mL (ready to use)	4°C
Plate Sealer	3 strips	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 235 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The concentrations can vary over a wide range depending on the different samples.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000 x g for 20 minutes at 4°C. Take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue samples: Weigh out 0.1 g tissue, homogenize with 1 mL of Assay Buffer on ice, centrifuged at 10,000 x g for 20 minutes at 4°C. Take the supernatant into a new centrifuge tube and keep it on ice for detection.

Liquid samples: detect it directly, or dilute with Assay Buffer.

ASSAY PROCEDURE

Each sample should be assayed in duplicate or triplicate.

Reagent	Sample tubes	Control tubes
Sample	10 µL	
Boiled Sample		10 µL
Substrate	90 µL	90 µL
Mix well and incubate for **15 minutes** at **50°C**.		
Stop Solution	100 µL	100 µL
Centrifuged at **5,000 x g** for **5 minutes**, then transfer **100 µL** in to the **96 Well UV Microplate**. Read the absorbance at **235 nm**.		

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Control and samples.
2. Unit Definition: **One unit** of **Pectate lyase activity** is defined as the enzyme **generates 1 nmol** of **Oligogalacturonic acid** per minute.:

   ε: molar extinction coefficient, 5.2×10^3 L/mol/cm;

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

   W: the weight of sample, g;

   $C_{Protein}$: the protein concentration, mg/mL;

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

   V_{Assay}: the volume of Assay Buffer, 1 mL;

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

   V_{Total}: the total volume of the enzymatic reaction, 0.2 mL;

   T: the reaction time, 15 minutes.

Formula:

According to the protein concentration of sample:

PL (U/mg)

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

$= 854.8 \times (OD_{Sample} - OD_{Control}) / C_{Protein}$

According to the weight of sample:

PL (U/g)

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

$= 854.8 \times (OD_{Sample} - OD_{Control}) / W$

According to the quantity of cells or bacteria

PL ($U/10^4$)

$= \{[(OD_{Sample} - OD_{Control}) / (\varepsilon \times d) \times V_{Total}] / (N \times V_{Sample} / V_{Assay})\} / T$

$= 854.8 \times (OD_{Sample} - OD_{Control}) / N$

According to the volume of sample

PL (U/ml)

$= \{[(OD_{Sample} - OD_{Control}) / (\varepsilon \times d) \times V_{Total}] / V_{Sample}\} / T$

$= 854.8 \times (OD_{Sample} - OD_{Control})$