(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Pectinase Assay Kit

Catalog No.: BC00167

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Pectinase Assay Kit
Detection Method	Colorimetric
Assay Type	Quantitative
Sample Type	Tissues, cells, cell culture medium, bacteria
Detection Instrument	Spectrophotometer (540 nm)

Product Introduction

Pectinase is a general term for a group of enzymes that degrade pectic substances, consisting of four major categories: protopectinase, pectinesterase, polygalacturonase and pectate lyase. It is widely found in plant fruits and microorganisms, and is mainly applied in the food, wine brewing, environmental protection, pharmaceutical, textile and daily chemical industries.

Principle

Pectinase hydrolyzes pectin to produce galacturonic acid, which contains reducing aldehyde groups. It reacts with DNS reagent to form a reddish-brown substance with a characteristic absorption peak at 540 nm. The pectinase activity can be calculated by measuring the change in absorbance at 540 nm.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	30 mL	Store at 4°C
Reagent 2	Powder	2 vials	Store at 4 °C. Add 12.5 mL Reagent 1 before use, heat at 50 °C to dissolve, and the solution can be stored for one week.
Reagent 3	Liquid	30 mL	Store at 4°C away from light
Reagent 4	Extraction solution	50 mL	Store at 4°C

Storage

The kit has a validity period of 3 months.

Preparation

Balance, temperature-controlled centrifuge, visible spectrophotometer, 1 mL glass cuvette (1 cm optical path), constant temperature water bath.

- **Sample handling**

1. Tissues: Homogenize tissues on ice at a mass-to-volume ratio of tissue (g) to extraction solution (mL) of 1:5-10 (it is recommended to weigh approximately 0.1 g tissue and add 1 mL extraction solution). Centrifuge the homogenate at 10000 g and 4 °C for 10 min, collect the supernatant and keep it on ice for subsequent measurement.
2. Bacteria and fungi: Lyse cells on ice by sonication at a cell count-to-volume ratio of cells (10^4 cells) to extraction solution (mL) of 500-1000:1 (it is recommended to add 1 mL extraction solution to 5 million cells). Sonication parameters: power 300 W, 3-second sonication with 7-second intervals, total sonication time 3 min. Afterwards, centrifuge at 10000 g and 4 °C for 10 min, collect the supernatant and keep it on ice for subsequent measurement.
3. Cell culture medium: Detect directly without pretreatment.

Operation process

	Control tube	Assay tube
Reagent 2 (mL)	0.4	0.4
Incubate in a 50 °C water bath for 5 min.		
Samples (mL)		0.1
Boiled samples (mL)	0.1	
Mix thoroughly and react in a 50 °C water bath for 30 min.		
Reagent 3 (mL)	0.5	0.5

Heat in a boiling water bath for 5 min, cool on ice to terminate the reaction, then centrifuge at 8000 g and 4 °C for 10 min. Collect the supernatant. Zero the spectrophotometer with distilled water at a wavelength of 540 nm using a 1 mL glass cuvette (1 cm optical path), and measure the absorbance value A of each tube.

Calculate $\Delta A = A_{Assay} - A_{Control}$.

Note: One sample tube and one control tube shall be prepared for each test sample. The solution of the control tube shall be treated in a boiling water bath before sampling and detection.

Calculation

1. Calculation based on protein concentration

Definition of enzyme activity: Under the conditions of 50 °C and pH 3.5, one unit (U) of enzyme activity is defined as the amount of enzyme that decomposes pectin to produce 1 mg galacturonic acid per milligram of protein per hour.

$$\text{Pectinase Activity (U/mg)} = \frac{\Delta A + 0.008}{3.9642} \times \frac{V_{Total}}{V_{Sample} \div Cpr} \div T$$

2. Calculated based on sample mass

Definition of enzyme activity: Under the conditions of 50 °C and pH 3.5, one unit (U) of enzyme activity is defined as the quantity of enzyme that degrades pectin to generate 1 mg galacturonic acid per gram of sample per hour.

$$\text{Pectinase Activity (U/g)} = \frac{\Delta A + 0.008}{3.9642} \times \frac{V_{Total}}{V_{Sample} \times W \div V} \div T$$

3. Calculation based on cell count

Definition of enzyme activity: Under the conditions of 50 °C and pH 3.5, one unit (U) of enzyme activity is defined as the amount of enzyme that degrades pectin to produce 1 mg galacturonic acid per 10^4 cells per hour.

$$\text{Pectinase Activity (U/}10^4\text{)} = \frac{\Delta A + 0.008}{3.9642} \times \frac{V_{Total}}{V_{Sample} \times \text{cell count} \div V} \div T$$

4. Cell culture medium

Definition of enzyme activity: Under the conditions of 50 °C and pH 3.5, one unit (U) of enzyme

activity is defined as the amount of enzyme that degrades pectin to produce 1 mg galacturonic acid per milliliter of culture medium per hour.

$$\text{Pectinase Activity (U/mL)} = \frac{\Delta A + 0.008}{3.9642} \times \frac{V_{Total}}{V_{Sample}} \div T$$

Annotation:

V_{Total}: Total reaction volume, 1 mL

V_{Sample}: Sample volume in reaction, 0.1 mL

V: Volume of extraction solution added, 1 mL

W: Sample mass, g

Cpr: Protein concentration of the test sample, mg/mL

T: reaction time, 0.5 h

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. If precipitate appears in Reagent 1, heat it at 50 °C to dissolve the sediment.
4. Perform a preliminary test before formal detection. If the absorbance value is excessively high or low, appropriately dilute the sample with extraction solution or increase the sample loading volume. Multiply the dilution factor in the calculation formula or adopt the actual sample volume added for calculation accordingly.
5. Boil the samples in boiling water for 10 minutes to completely inactivate enzymes.