CheKine™ Micro Pectinase Activity Assay Kit

Cat #: KTB1581 Size: 48 T/24 S 96 T/48 S

	Micro Pectinase Activity Assay Kit		
REF	**Cat #:** KTB1581	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant tissues, Bacteria, Fungi, Cell culture media or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Pectinase is a general term for a class of enzymes that decompose pectin, including polygalacturonase, pectin esterase, pectin lyase, and pectin methylesterase. These enzymes are widely found in plant fruits and microorganisms and are primarily used in industries such as food, winemaking, environmental protection, pharmaceuticals, textiles, and daily chemical products. CheKine™ Micro Pectinase Activity Assay Kit offers a simple, convenient, and rapid approach for assessing sucrase activity, which is suitable for plant tissues, bacteria, fungi, cell culture media or other Liquid samples. The principle of the kit is based on the hydrolysis of pectin by pectinase to produce galacturonic acid, which has a reducing aldehyde group. This reacts with DNS reagent to form a reddish-brown substance that has a characteristic absorbance peak at 540 nm. Measuring the changes in absorbance at 540 nm allows for the calculation of pectinase activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	120 mL	120 mL×2	4℃
Reagent Ⅰ	12 mL	24 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Reagent Ⅲ	12 mL	24 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- Water bath, analytical balance, ice maker, low-temperature centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water

• Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent II: Prepared before use. Take one bottle of Reagent II and add 10.5 mL of Reagent I. Heat the mixture to 50℃ to dissolve completely. Store any unused portion of the reagent at 4℃ in the dark for up to one week.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Note: Reagent III is toxic, so it is recommended to experiment in a fume hood.

Standard: Prepared before use. Dissolve by adding 0.978 mL of deionized water to make a 50 μmol/mL galacturonic acid solution, and reserve for later use. This solution can be stored in the dark at 4℃ for up to one week.

Standard preparation: Using 50 μmol/mL galacturonic acid solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (μmol/mL)
Std.1	100 μL 50 μmol/mL Standard	400	10
Std.2	80 μL 50 μmol/mL Standard	420	8
Std.3	60 μL 50 μmol/mL Standard	440	6
Std.4	40 μL 50 μmol/mL Standard	460	4
Std.5	20 μL 50 μmol/mL Standard	480	2
Std.6	10 μL 50 μmol/mL Standard	490	1
Std.7	5 μL 50 μmol/mL Standard	495	0.5
Std.8	0	500	0 (Blank Tube)

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Plant Tissue: Weigh approximately 0.1 g of the sample, and add 1 mL of Extraction Buffer to homogenize in an ice bath. Centrifuge at 10,000 g and 4℃ for 10 min, and retain the supernatant on ice for analysis.

2. Bacteria or fungi: Collect 5×10^6 bacteria or fungi into the centrifuge tube, wash bacteria or fungi with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the bacteria or fungi 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3. Cell culture media or other Liquid samples: Test directly.

Note: 1. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

2. For plant fruit tissue samples, it is recommended to dilute the samples 10 to 20 times with Extraction Buffer before measurement.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm. Visible

spectrophotometer was returned to zero with deionized water.

2. Take 40 μL of the sample and boil in a water bath for 10 min; set aside for later use.

3. Operation table (The following operations are operated in a 1.5 mL EP tube):

Reagent	Control Tube (μL)	Test Tube (μL)	Standard Tube (μL)
Working Reagent II	120	120	120
Incubate in a 50℃ water bath for 5 min			
Sample	0	30	0
Boiled sample	30	0	0
Standard	0	0	30
Mix well and incubate in a 50℃ water bath for 30 min			
Reagent III	150	150	150
Boil in a water bath for 5 min, then cool in an ice bath to stop the reaction. Centrifuge at 8,000 g at 4℃ for 10 min, and adjust the zero point with distilled water. Transfer 200 μL of the supernatant to a microglass cuvette or a 96-well plate, then measure the absorbance at 540 nm, recording the values as $A_{Control}$, $A_{Test,}$, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.			

Note: The standard curve and Blank Tube need to be measured only 1-2 times, while each Test Tube requires a corresponding Control Tube. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA_{Test} is less than 0.05, the sample volume can be appropriately increased. If ΔA_{Test} is greater than 1.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. Maintain consistent cooling times for each experiment.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation, and bring the ΔA_{Test} into the equation to get the x value (μmol/mL).

2. Calculation of pectinase activity:

(1)Calculated by protein concentration

Unit Definition: the amount of enzyme that catalyzes the hydrolysis of pectin to produce 1 μmol of galacturonic acid per h per mg of protein at 50℃ and pH 3.5.

Pectinase (U/mg prot)=$x\times V_{Total}\div(V_{Sample}\times Cpr\div V_{Total\ Sample})\div T\times F$**$=10\times x\div Cpr\times F$**

(2)Calculated by sample weight

Unit Definition: the amount of enzyme that catalyzes the hydrolysis of pectin to produce 1 μmol of galacturonic acid per h per g of tissue at 50℃ and pH 3.5.

Pectinase (U/g weight)=$x\times V_{Total}\div(V_{Sample}\times W\div V_{Total\ Sample})\div T\times F$**$=10\times x\div W\times F$**

(3) Calculated by bacteria or fungi number

Unit Definition: the amount of enzyme that catalyzes the hydrolysis of pectin to produce 1 μmol of galacturonic acid per h per 10^4 of bacteria or fungi at 50℃ and pH 3.5.

Pectinase ($U/10^4$)=$x\times V_{Total}\div(V_{Sample}\times N\div V_{Total\ Sample})\div T\times F$**$=10\times x\div N\times F$**

(4) Calculated by volume of liquid samples

Unit Definition: the amount of enzyme that catalyzes the hydrolysis of pectin to produce 1 μmol of galacturonic acid per h per mL

of liquid at 50℃ and pH 3.5.

Pectinase (U/mL)=x×V_{Total}÷V_{Sample}÷T×F**=10×x×F**

Where: V_{Total}: total reaction volume, 0.15 mL; V_{Sample}: sample volume added, 0.03 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr; sample protein concentration, mg/mL; W: sample weight, g; T: reaction time, 0.5 h; N: total number of bacteria or fungi, 10^4; F: sample dilution factor.

Typical Data

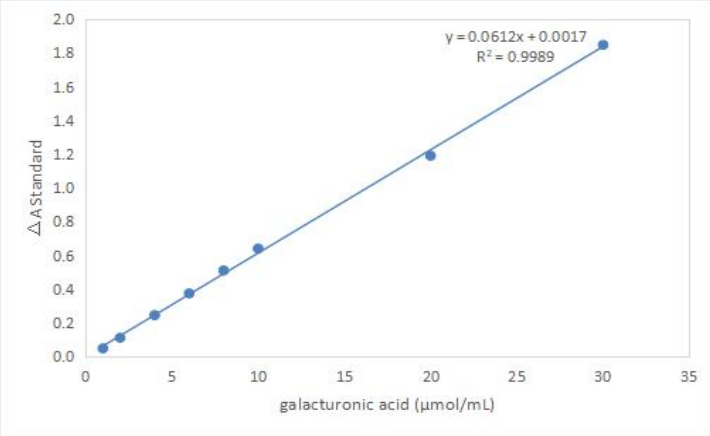

Figure 1. Standard curve of galacturonic acid

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.