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CheKine™ Mirco Pectin Lyase (PL) Activity Assay Kit

Cat #: KTB1582 Size: 48 T/96 T

	Mirco Pectin Lyase (PL) Activity Assay Kit		
REF	**Cat #:** KTB1582	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues, Bacteria, Fungi and Culture media		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Pectin lyase (EC4.2.2.10) is an important component of pectinases, which is a depolymerase that can degrade plant cell walls, leading to the softening or even death of plant tissues. It has a wide range of sources, mainly from microorganisms. It can be used for clarification of fruit juice and wine, improving the yield of fruit juice, purification of plant viruses, bleaching of pulp, and biorefining of textiles. It has potential application value in reducing environmental pollution and lowering energy consumption. The CheKine™ Mirco Pectin Lyase Activity Assay Kit offers a simple, convenient, and rapid method for detecting PL activity, suitable for samples such as plant tissues, bacteria, fungi, and culture media. Its principle involves pectin lyase acting on the α-1,4-glycosidic bonds in pectin to produce unsaturated oligogalacturonic acids with an unsaturated bond between C4 and C5 at the reducing end, which have a characteristic absorption peak at 235 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent I	3 mL	6 mL	4℃, protected from light
Reagent II	3 mL	6 mL	4℃, protected from light
Reagent III	3 mL	6 mL	4℃

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 235 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, analytical balance, ice maker, centrifuge
- 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℃, protected from light.
Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

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 tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
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. Culture Media: Test directly.

Note: 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.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 235 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 96-well UV plate or microquartz cuvette)

Reagent	Control Well (μL)	Test Well (μL)
Reagent I	0	120
Reagent II	120	0
Incubate at 40℃ for 3 min		
Sample	20	20
Mix well and react at 40℃ for 30 min		
Reagent III	60	60
Mix well, and measure the absorbance at 235 nm for both $A_{Control}$ and A_{Test} in a 96-well UV plate or a micro quartz cuvette. Calculate $\Delta A=A_{Test}-A_{Control}$.		

Note: Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment, each test well should have a corresponding control well. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If A_{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.

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.

A. 96-well UV plates calculation formula as below

1. Calculated by protein concentration

Active unit definition: One unit of enzyme activity is defined as the amount of enzyme required to hydrolyze pectin to produce 1 nmol of unsaturated galacturonic acid per min per mg of protein at 40℃ and pH 5.5.

PL (U/mg prot)=ΔA÷(ε×d)×V_{Total}÷(V_{Sample}×Cpr)÷T**=128.2×ΔA÷Cpr**

2. Calculated by sample fresh weight

Active unit definition: One unit of enzyme activity is defined as the amount of enzyme required to hydrolyze pectin to produce 1 nmol of unsaturated galacturonic acid per min per g of tissue at 40℃ and pH 5.5.

PL (U/mg prot)=ΔA÷(ε×d)×V_{Total}÷(V_{Sample}×W÷$V_{Total\ Sample}$)÷T**=128.2×ΔA÷W**

3. Calculated by bacteria or fungi number

Active unit definition: One unit of enzyme activity is defined as the amount of enzyme required to hydrolyze pectin to produce 1 nmol of unsaturated galacturonic acid per min in 10^4 bacteria or fungi at 40℃ and pH 5.5.

PL ($U/10^4$)=ΔA÷(ε×d)×V_{Total}÷(V_{Sample}×F÷$V_{Total\ Sample}$)÷T**=128.2×ΔA÷F**

4. Calculated by culture media volume

Active unit definition: One unit of enzyme activity is defined as the amount of enzyme required to hydrolyze pectin to produce 1 nmol of unsaturated galacturonic acid per min per mL of culture media at 40℃ and pH 5.5.

PL (U/mL)=ΔA÷(ε×d)×V_{Total}÷V_{Sample}÷T**=128.2×ΔA**

Where: ε: Molar extinction coefficient of unsaturated galacturonic acid, 5,200 L/mol/cm; d: 96-well plate diameter, 0.5 cm; V_{Total}: total reaction volume, 0.2 mL; V_{Sample}: sample volume added, 0.02 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; W: sample weight, g; T: reaction time, 30 min; F: Total number of bacteria or fungi, 10^4.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Precautions

1. It is recommended not to measure too many samples at once to avoid excessive delays in the enzymatic reaction times.

Typical Data

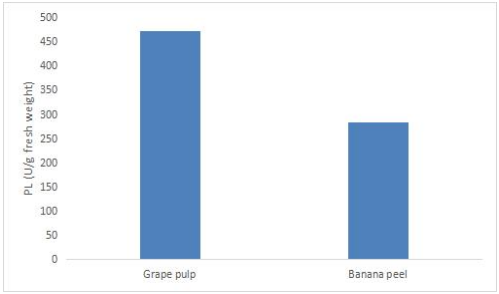

Figure 1. Determination PL activity in Grape pulp and Banana peel by this assay kit

Recommended Products

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

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.