CheKine™ Micro Ploygalacturonase (PG) Activity Assay Kit

Cat #: KTB1333 Size: 48 T/96 T

	Micro Ploygalacturonase (PG) Activity Assay Kit		
REF	**Cat #:** KTB1333	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissue, Bacteria, Fungus, Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Ploygalacturonase (PG) (EC 3.2.1.15) is a kind of pectinase, which is widely found in plants, bacteria and fungi. It catalyses the decomposition of polygalacturonic acid, which plays an important role in fruit softening, pollen pollination, seed maturation and organ shedding. In addition, when pathogenic bacteria infect host plants, they can secrete polygalacturonase to degrade host cell wall, thus leading to the development of disease. CheKine™ Micro Ploygalacturonase (PG) Activity Assay Kit can be used to detect biological samples such as plant tissue, bacteria,fungus, liquid samples. In the kit, PG hydrolyzes polygalacturonic acid to produce galaturonate, which has a reducing aldehyde group, reacts with DNS reagent to produce reddish-brown substance, and has a characteristic absorption peak at 540 nm. The activity of polygalacturonase can be calculated by measuring the change of absorption value at 540 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	12 mL	24 mL	4℃
Reagent Ⅱ	15 mL	30 mL	4℃, protected from light
Standard	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water
- Homogenizer or mortar (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℃.

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

Standard: Prepared before use; Add 0.978 mL deionized water to fully dissolve, that is 50 μmol/mL galaturonate standard; Store at 4℃, protected from light for 2 weeks. Use the 50 μmol/mL galaturonate standard solution and further dilute it to the standard as shown in the following table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (μmol/mL)
Std.1	200 μL of 50 μmol/mL Standard	800	10
Std.2	160 μL of Std.1 (10 μmol/mL)	40	8
Std.3	120 μL of Std.1 (10 μmol/mL)	80	6
Std.4	80 μL of Std.1 (10 μmol/mL)	120	4
Std.5	40 μL of Std.1 (10 μmol/mL)	160	2
Blank	0	200	0

Notes: Always prepare fresh Standards per use; Diluted Std. 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 Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice. Centrifuge at 16,000 g for 10 min at 4℃. Use supernatant for assay.
2. Bacteria or Fungus: Collect 5×10^6 bacteria or fungus into the centrifuge tube, wash bacteria or fungus with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the bacteria or fungus 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 16,000 g for 10 min at 4℃. Use supernatant for assay.
3. Liquid: Test directly.

Note: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Cat #: KTD3002, if it is calculated by protein concentration.

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. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Sample	0	0	30	0
Inactivated Samples (water bath at 95℃ for 10 min)	0	0	0	30
Standard	0	30	0	0
Deionized Water	30	0	0	0

Reagent Ⅰ	120	120	120	0
After accurate reaction in 40℃ water bath for 2 h, heat in 95℃ water bath for 10 min (cover tightly to prevent water loss), and cool to room temperature naturally after removal.				
Reagent Ⅰ	0	0	0	120
Reagent Ⅱ	150	150	150	150

3. Mix well, water bath at 95℃ for 5 min (cover tightly to prevent water loss), natural cooling to room temperature, take 200 μL into a 96-well plate or microglass cuvette. Detect the absorbance at 540 nm. The Blank Well is recorded as A_{Blank}, the standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}, the Control Well is marked as $A_{Control}$. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.1, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.8, 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.

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 and obtain the standard equation. The determination of ΔA_{Test} is brought into the equation to get x (μmol/mL).

2. Calculation of the PG activity

(1) Calculated by protein concentration

Active unit definition: At 40℃, pH6.0, 1 μmol galaturonate is produced by hydrolyzing polygalacturonic acid per h in 1mg tissue protein reaction system is defined as a unit of enzyme activity.

PG (U/mg prot)=$(V_{Sample}\times x)\div(V_{Sample}\times Cpr)\div T$**=0.5x÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: At 40℃, pH6.0, 1 μmol galaturonate is produced by hydrolyzing polygalacturonic acid per h in 1 g tissue reaction system is defined as a unit of enzyme activity.

PG (U/g fresh weight)=$(V_{Sample}\times x)\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**=0.5x÷W**

(3) Calculated by bacteria or fungus

Active unit definition: At 40℃, pH6.0, 1 μmol galaturonate is produced by hydrolyzing polygalacturonic acid per h in 10^4 bacteria or fungus reaction system is defined as a unit of enzyme activity.

PG ($U/10^4$)=$(V_{Sample}\times x)\div(n\times V_{Sample}\div V_{Total\ sample})\div T$**=0.5x÷n**

(4) Calculated by volume of liquid samples

Active unit definition: At 40℃, pH6.0, 1 μmol galaturonate is produced by hydrolyzing polygalacturonic acid per h in 1 mL liquid samples reaction system is defined as a unit of enzyme activity.

PG (U/mL)=$(V_{Sample}\times x)\div(V_{Sample}\div V_{Total\ sample})\div T$**=0.5x**

V_{Sample}: Added the sample volume, 30 μL; $V_{Total\ sample}$: Added the Extraction Buffer volume, 1 mL;T: Reaction time, 2 h; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; n: Number of bacteria or fungus, calculated in units of ten thousand.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

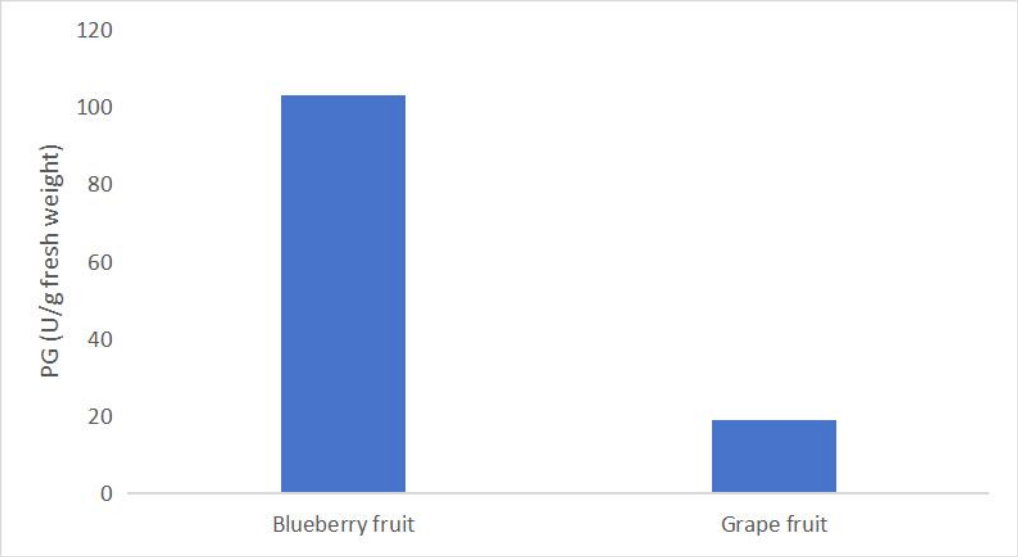

Figure 1. Determination of PG activity in blueberry fruit and grape fruit by this kit.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Content Assay Kit

Disclaimer

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