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

Catalog No：E-BC-K867-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（530-550 nm）

Detection range：0.05-20 U/mL

Elabscience® Pectinase Activity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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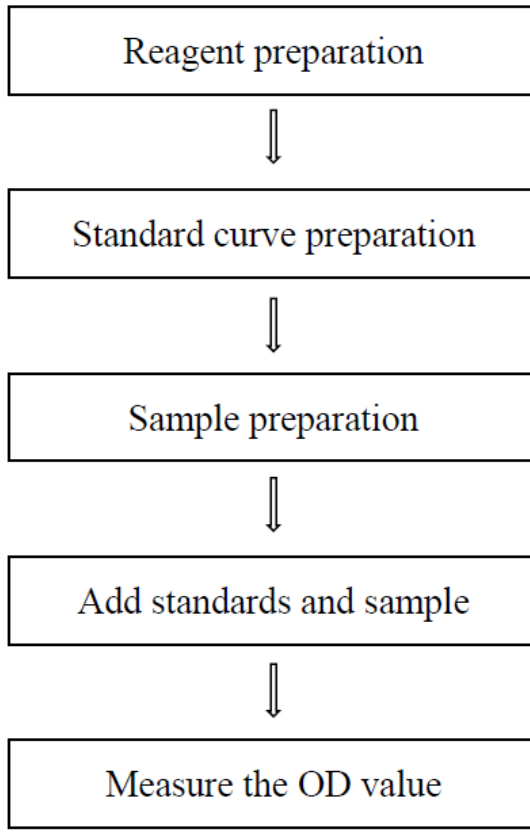
Assay summary



Intended use

This kit can measure pectinase activity in liquid, plant tissue and microorganism samples.

Detection principle

Pectinase is a class of pectin decomposition enzymes, including propectinase, pectin esterase, polygalacturonase and pectin lyase, which widely exist in plant fruits and microorganisms, and is the most important enzyme in fruit processing.

The detection principle of this kit is that the substance generated by the hydrolysis of pectin by pectinase reacts with the chromogenic agent to produce a brown-red substance with characteristic absorption peak at 540 nm. The activity of pectinase can be calculated by measuring the change of absorbance value at 540 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	40 mL ×1 vial	2-8°C, 12 months
Reagent 2	Substrate	Powder ×1 vial	2-8°C, 12 months
Reagent 3	Buffer Solution	40 mL ×1 vial	2-8°C, 12 months
Reagent 4	Chromogenic Agent	40 mL ×1 vial	2-8°C, 12 months
Reagent 5	Standard	Powder ×1 vial	2-8°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (530-550 nm, optimum wavelength: 540 nm)， Water bath, Ultrasonic cell disruptor

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of working solution：

Dissolve one vial of substrate with 40 mL of buffer solution, mix well to dissolve in a 50 ℃ water bath (oscillate 3-5 times). Aliquoted storage at -20 ℃ for 2 months, and avoid grown bacteria.

③ The preparation of 50 μmol/mL standard solution：

Dissolve one vial of standard with 0.943 mL of double distilled water, mix well to dissolve. Store at 2-8 ℃ for a week.

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 50 μmol/mL standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 3, 4, 6, 8, 10 μmol/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/mL)	**0**	**1**	**2**	**3**	**4**	**6**	**8**	**10**
50 μmol/mL Standard (μL)	0	4	8	12	16	24	32	40
Double distilled water (μL)	200	196	192	188	184	176	168	160

Sample preparation

Liquid samples: Test directly, samples can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of plant tissue needed for each assay (initial recommendation 50 mg).

② Homogenize 50 mg tissue in 450 μL of extraction solution with a dounce homogenizer.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M).

Microorganism samples:

① Harvest the number of bacteria needed for each assay (initial recommendation 2.5×10^6 bacteria).

② Wash bacteria with PBS (0.01 M, pH 7.4).

③ Homogenize 2.5×10^6 bacteria in 500 μL extraction solution on ice bath ultrasonic crushing bacteria (power 300 w, ultrasonic 3 s, interval 7 s, total time 3 min).

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

The preparation of sample in control tube： Take 40 μL sample in boiling water bath for 10 min.

The key points of the assay

① If the OD_{sample} is more than 1.5, the sample should be diluent with extraction solution.

② For the plant fruit tissue, it is recommended to dilute the sample with extraction solution 5-20 times before determination.

Operating steps

① Add 200 μL of working solution into each tube.

② Incubate in water bath at 50 ℃ for 5 min.

③ Standard tube: Add 40 μL of standard solution with different concentrations into standard tubes.

Sample tube: Add 40 μL of sample into sample tubes.

Control tube: Add 40 μL of boiling sample into control tubes.

④ Vortex mixing and reaction in 50 ℃ water bath for 30 min. Immediately boiling water bath for 5 min, and cool to room temperature. Centrifuge at 8000×g for 10 min at 25℃ and collect supernatant.

⑤ Add 150 μL of supernatant and 150 μL of chromogenic agent into 1.5 mL EP tubes. Boiling water bath for 5 min, ice bath cooling to terminate the reaction. Take 200 μL on the microplate and measure the OD values of each well at 540 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Liquid samples:

Definition: At 50 ℃ and pH = 3.5, the amount of enzyme in 1 mL sample that decomposing pectin to produce 1 μmol galacturonic acid in 1 hour is defined as 1 unit.

$$\underset{\text{(U/mL)}}{\text{pectinase activity}} = (\Delta A_{540} - b) \div a \div T \times f$$

2. Tissue sample:

Definition: At 50 ℃ and pH = 3.5, the amount of enzyme in 1 mg tissue protein that decomposing pectin to produce 1 μmol galacturonic acid in 1 hour is defined as 1 unit.

$$\underset{\text{(U/mgprot)}}{\text{pectinase activity}} = (\Delta A_{540} - b) \div a \div T \div C_{pr} \times f$$

3. Bacteria sample:

Definition: At 50 ℃ and pH = 3.5, the amount of enzyme in 10^6 bacteria that decomposing pectin to produce 1 μmol galacturonic acid in 1 hour is defined as 1 unit.

$$\underset{\text{(U/10\^6)}}{\text{pectinase activity}} = (\Delta A_{540} - b) \div a \div n \div T \times V \times f$$

[Note]

ΔA_{540}: $\Delta A_{540} = OD_{Sample} - OD_{control}$.

m： The wet weight of sample, g.

V： The volume of extraction solution, 0.5 mL.

n: The number of bacteria sample/10^6.

T: Reaction time, 0.5 h.

f： Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, mgprot/mL.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	7.50	10.00	15.00
%CV	4.8	4.1	3.5

Inter-assay Precision

Three rat serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	7.50	10.00	15.00
%CV	6.6	6.1	4.5

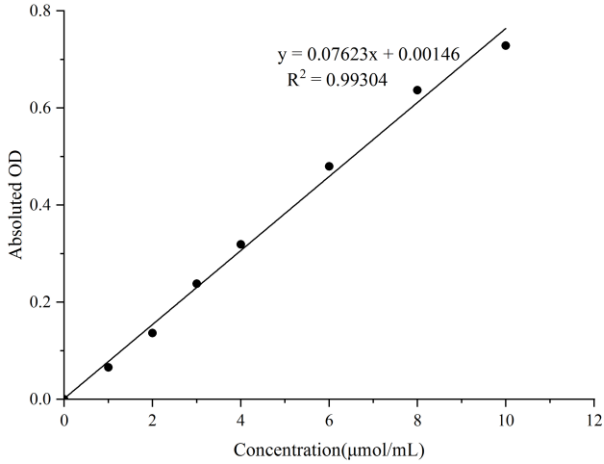
Sensitivity

The analytical sensitivity of the assay is 0.05 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/mL)	**0**	**1**	**2**	**3**	**4**	**6**	**8**	**10**
OD value	0.111	0.177	0.249	0.349	0.430	0.592	0.746	0.840
	0.112	0.177	0.247	0.350	0.431	0.590	0.750	0.840
Average OD	0.112	0.177	0.248	0.350	0.431	0.591	0.748	0.840
Absoluted OD	0.000	0.066	0.137	0.238	0.319	0.480	0.637	0.729



Appendix II Example Analysis

Example analysis:

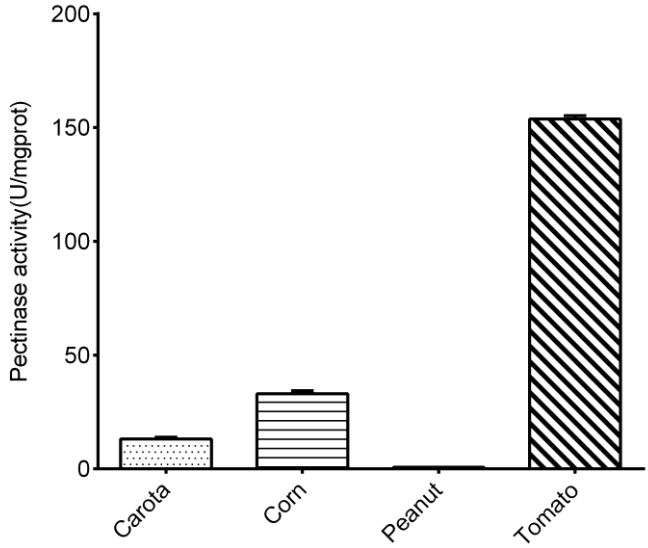
Take 40 μL of 10% tomato tissue homogenization which dilute for 5 times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.07623 x + 0.00146. The OD value of sample is 0.478, the OD value of control is 0.366, the concentration of sample protein is 0.095 mgprot/mL and the calculation result is:

$$\text{pectinase activity(U/mgprot)} = (0.478 - 0.366 - 0.00146) \div 0.07623 \div 0.095 \times 5 \div 0.5$$

$$= 152.64 \text{ U/mgprot}$$

Detect 10% carota tissue homogenization (the concentration of protein is 0.196 mgprot/mL), 10% corn tissue homogenization (the concentration of protein is 0.091 mgprot/mL), 10% peanut tissue homogenization(the concentration of protein is 2.125 mgprot/mL) and 10% tomato tissue homogenization(the concentration of protein is 0.095 mgprot/mL, dilute for 5 times), according to the protocol, the result is as follows:



Statement

1. This assay kit is for research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.