（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：0.15-20 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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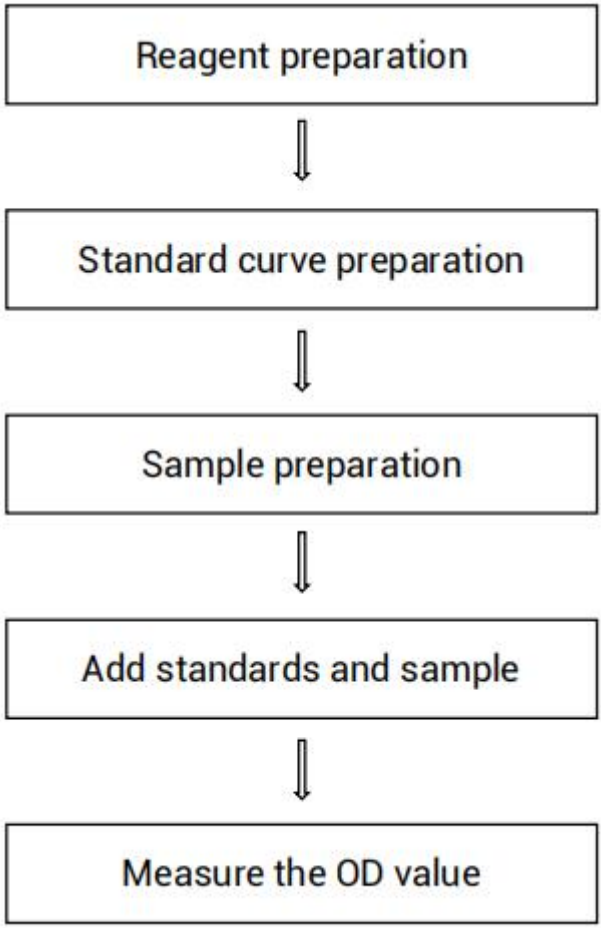
Assay summary



Intended use

This kit can be used to measure pectinase activity in plant tissue samples.

Detection principle

Pectinase is an enzyme that breaks down pectin. Depending on the different substrates it acts upon, it can be classified into three types: pectin esterase, polygalacturonic acidase and pectin lyase. It is widely present in higher plants and microorganisms and is widely used in the food industry.

The detection principle of this kit: The substances generated by pectinase hydrolyzing pectin react with the chromogenic agent upon heating to form a brown-colored substance. The activity of pectinase is calculated by measuring the change in absorbance at 540 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	60 mL × 2 vials	2-8℃,12months
Reagent 2	Substrate	Power × 1 vial	2-8℃, 12 months, shading light
Reagent 3	Buffer Solution	25 mL × 1 vial	2-8℃, 12 months
Reagent 4	Chromogenic Agent	20 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 5	Standard	Power × 1 vial	2-8℃, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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), Incubator, Water bath

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② It is a normal phenomenon which yellow precipitate is observed to form in chromogenic agent. Heat in a 70°C water bath for 10 min, then cool naturally to 25°C before use.

③ The preparation of substrate working solution：
Dissolve one vial of substrate with 25 mL of buffer solution, mix well to dissolve. Heat in a 50°C water bath for 15 min (shock 3-5 times during the period). Aliquoted storage at -20°C for 1 month.

④ The preparation of 50 μmol/mL standard solution：
Dissolve one vial of standard with 1 mL of extraction solution, mix well to dissolve. Store at 2-8°C for 2 weeks.

⑤ The preparation of 10 μmol/mL standard solution：
Before testing, please prepare sufficient 10 μmol/mL standard solution. For example, prepare 1000 μL of 10μmol/mL standard solution (mix well 200 μL of 50 μmol/mL standard solution and 800 μL of extraction solution). Store at 2-8°C for 3 days.

⑥ The preparation of standard curve：

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

Dilute 10 μmol/mL standard solution with extraction solution 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**
10 μmol/mL standard (μL)	0	20	40	60	80	120	160	200
Extraction Solution (μL)	200	180	160	140	120	80	40	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C. Collect supernatant and keep it on ice for detection. Detect the prepared sample within 8 h.

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

Control sample：

Take a portion of the supernatant and heat it in boiling water for 5 min (There might be some precipitates after the samples in the boiling water bath, but no need for centrifugation, just mix well).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Tomato pulp tissue homogenate	5-10
10% Navel orange pulp tissue homogenate	5-10
10% Banana peel tissue homogenate	2-10
10% Apple pulp tissue homogenate	10-20
10% Navel orange peel tissue homogenate	10-20
10% Corn seed tissue homogenate	2-5

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

When the OD value of the sample well is greater than 1.8, it is recommended to dilute the sample with extraction solution.

Operating steps

① Standard tube: add 50 μL of standard solution with different concentrations to the tubes.
Sample tube: add 50 μL of sample into the tubes.
Control tube: add 50 μL of control sample into the tubes.

② Add 200 μL of substrate working solution into the tubes.

③ Mix well, water bath at 50°C for 30 min.

④ Immediately terminate the reaction in boiling water bath for 5 min and then cool it with running water.

⑤ Centrifuge at 8000×g for 5 min at 25°C for sample tubes and control tubes, collect supernatant for detection.

⑥ Take 150 μL of supernatant for each tube into new tubes, add 150 μL of chromogenic agent.

⑦ Boiling water bath for 5 min for each tube, cool it with running water and mix well. Take 200 μL of solution to the microplate and measure the OD value 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. Calculated for tissue weight:

Definition: The amount of enzyme in 1 g tissue per 1 h that resolve pectinase of 1 μmol galacturonic acid at 50℃, pH = 3.5 is defined as 1 unit.

$$\frac{\text{Pectinase activity}}{\text{(U/g wet weight)}} = (\Delta A - b) \div a \div T \times f \div \frac{m}{V}$$

2. Calculated for protein concentration:

Definition: The amount of enzyme in 1 g tissue protein per 1 h that resolve pectinase of 1 μmol galacturonic acid at 50℃, pH = 3.5 is defined as 1 unit

$$\frac{\text{Pectinase activity}}{\text{(U/mgprot)}} = (\Delta A - b) \div a \div T \times f \div C_{pr}$$

[Note]

ΔA: $OD_{Sample} - OD_{Control}$.

f: Dilution factor of sample before test.

m: The wet weight of tissue, g.

V: The volume of extraction solution, mL.

T: Reaction time, 0.5 h.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 10% corn seed tissue homogenate 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)	5.0	10.0	15.0
%CV	1.1	1.4	1.1

Inter-assay Precision

Three 10% corn seed tissue homogenate 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)	5.0	10.0	15.0
%CV	7.2	3.6	4.1

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	5.0	10.0	15.0
Observed Conc. (U/L)	4.65	10.00	15.10
Recovery rate (%)	93	100	101

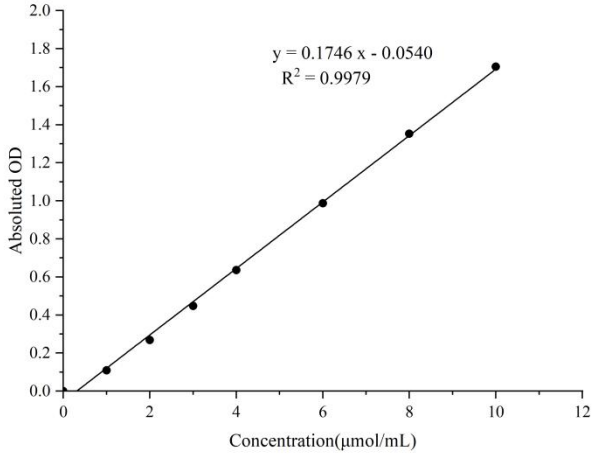
Sensitivity

The analytical sensitivity of the assay is 0.15 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	0.105	0.214	0.373	0.556	0.746	1.099	1.460	1.822
	0.107	0.215	0.375	0.550	0.737	1.087	1.458	1.799
Average OD	0.106	0.215	0.374	0.553	0.742	1.093	1.459	1.811
Absoluted OD	0.000	0.109	0.268	0.447	0.636	0.987	1.353	1.705



Appendix Π Example Analysis

Example analysis：

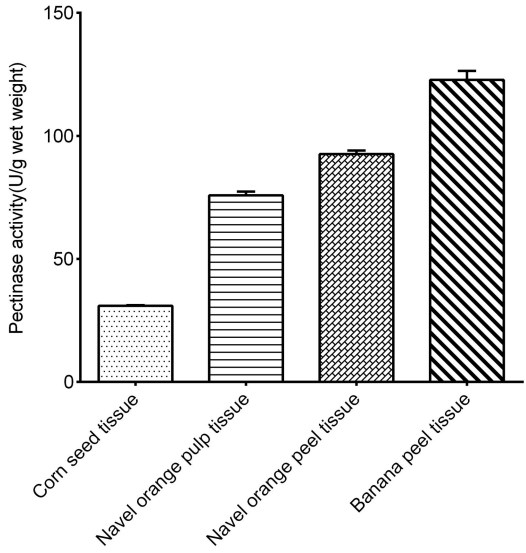
Take 50 μL of 10% corn seed tissue homogenate (dilute for 2 times) into the well, and carry the assay according to the operation steps. The results are as follows:

The standard curve is y = 0.1746 x - 0.0540, the OD value of the sample well is 0.721, the OD value of the control well is 0.625, and the calculation result is:

Pectinase activity (U/g wet weight) = (0.721 - 0.625 + 0.054) ÷ 0.1746 ÷ 0.5

× 2 ÷ 0.1 ×0.9 = 30.93 U/g wet weight

Detect 10% corn seed tissue homogenate (dilute for 2 times), 10% navel orange pulp tissue homogenate (dilute for 10 times), 10% navel orange peel tissue homogenate (dilute for 10 times) and 10% banana peel tissue homogenate (dilute for 10 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.