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

Catalog No：E-BC-K1223-M

Specification：96T (40 samples)

Measuring instrument：Microplate reader (530-550 nm)

Detection range：6.22-333.33 U/mL

Elabscience® β-1, 3-Glucanase 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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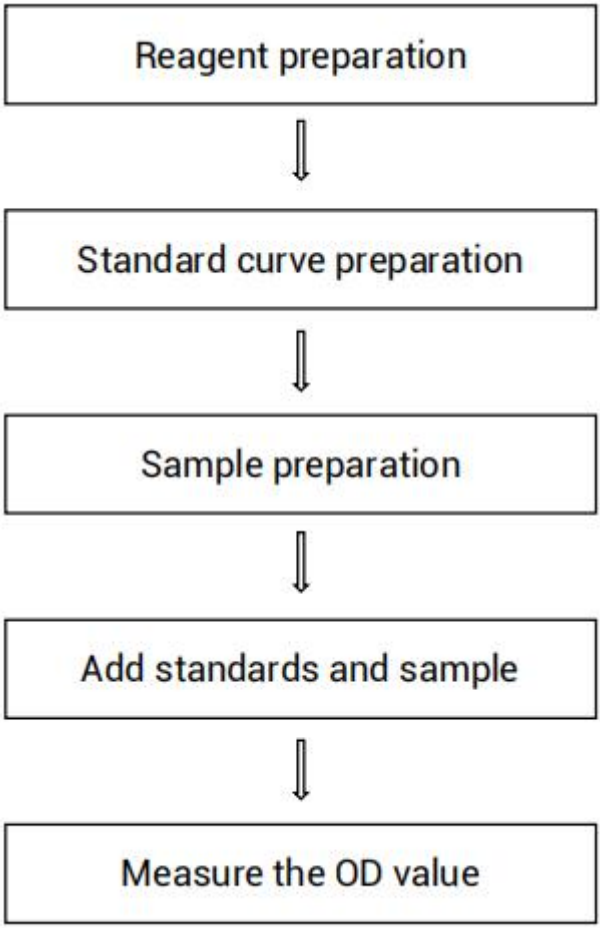
Assay summary



Intended use

This kit can be used to measure β-1, 3-glucanase activity in plant tissue samples.

Detection principle

β-1, 3-glucanase (β-1,3-GA, EC 3.2.1.39) is mainly found in plants and catalyzes the hydrolysis of β-1, 3-glucosidic bonds, thereby destroying fungal cell walls. In particular, when synergistic with chitinase, it can significantly inhibit the growth of fungi. When plants are infected with diseases or under other stress conditions, they can induce a large amount of β-1, 3-GA to enhance plant resistance to adverse external stimuli. Therefore, the determination of β-1, 3-GA activity is widely used in plant pathology and stress physiology research.
β-1,3-GA hydrolyses the β-1, 3-glucosidic linkage of laminarin to produce reducing ends. The activity can be reflected by measuring the amount of reducing sugar using 3, 5-dinitrosalicylic acid.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	120 mL × 1 vial	2-8°C, 12 months
Reagent 2	Buffer Solution	8 mL × 1 vial	2-8°C, 12 months
Reagent 3	Substrate	Powder × 1 vial	2-8°C, 12 months
Reagent 4	Chromogenic Agent	60 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 5	Standard	Powder × 1 vial	2-8°C, 12 months
	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), Homogenizer, Water bath, Centrifuge, Incubator (37℃)

Reagents:

95% alcohol, 80% alcohol

Reagent preparation

① Equilibrate the buffer solution, substrate, chromogenic agent and standard to 25℃ before use.

② The preparation of substrate working solution:
Dissolve one vial of substrate with 4.5 mL of buffer solution, mix well to dissolve. Aliquoted storage at 2-8°C for 2 weeks.

③ The preparation of 10 mg/mL standard solution:
Dissolve one vial of standard with 1 mL of double distilled water, mix well to dissolve. Aliquoted storage at -20°C for 2 days.

④ The preparation of standard curve:

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

Dilute 10 mg/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 mg/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	**0**	**1**	**2**	**3**	**4**	**6**	**8**	**10**
10 mg/mL Standard (μL)	0	10	20	30	40	60	80	100
Double distilled water (μL)	100	90	80	70	60	40	20	0

The key points of the assay

① When ΔA ($OD_{sample} - OD_{control}$) ≤ 0.005, it is recommended to extend the incubation time at 37°C in the operating steps, or increase the concentration of tissue homogenate.

② Please precool the 95% ethanol, 80% ethanol and extraction solution to 2-8°C before use.

Sample preparation

① Sample preparation

Tissue sample:

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

② Homogenate 200 mg tissue in 1 mL of precooled 95% ethanol with a dounce homogenizer at 4°C. Stand it at 4°C for 10 min.

③ Centrifuge at 12000×g for 5 min at 4℃ to remove supernatant and collect the precipitate.

④ Add precooled 1 mL of 80% ethanol, and mix fully. Stand it at 4°C for 10 min. Centrifuge at 12000×g for 5 min at 4℃ to remove the supernatant and collect the precipitate.

⑤ Add precooled 1 mL of extraction solution and mix fully. Stand it at 4°C for
10 min. Centrifuge at 12000×g for 10 min at 4℃. Collect supernatant and keep it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
20% Epipremnum aureum tissue homogenate	1
20% Corn kernel tissue homogenate	1
20% Sweet potato tissue homogenate	1

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

Operating steps

Control samples: Take 0.2 mL of the sample supernatant to the new EP tubes, incubate it in a boiling water bath for 15 min, cooled with running water. Centrifuge at 12000×g for 10 min at 4℃, and collect the supernatant as a control sample.

① Standard tube: Add 20 μL of standard with different concentrations to the corresponding tubes.
Control tube: Add 20 μL of control samples to the corresponding tubes.
Sample tube: Add 20 μL of samples to the corresponding tubes.

② Add 20 μL of double distilled water to the standard tubes.
Add 20 μL of substrate working solution to the sample tubes and control tubes.

③ Mix fully and incubate at 37°C for 30 min.

④ Add 300 μL of chromogenic agent to each tube.

⑤ Mix fully, water bath at 95°C for 5 min (cover tight to prevent water loss) then cool to 25℃ with running water.

⑥ Add 560 μL of double distilled water to each tube.

⑦ Mix fully and take 200 μL of solution to each tube to the microplate. Measure the OD value of each well at 540 nm with microplate reader, $\Delta A = A_{sample} - A_{control}$.

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:

For tissue samples:

Definition: The amount of enzyme in 1 g tissue per 1 min that consume laminarin to produce 1 μg of reducing sugar at 37°C is defined as 1 unit.

$$\text{β-1,3-GA activity (U/g wet weight)} = (\Delta A - b) \div a \times 10^3 \times V_1 \times f \div (m \times V_1 \div V_2) \div T$$

$$= 33.33 \times (\Delta A - b) \div a \div m \times V_2 \times f$$

[Note]

ΔA: $OD_{sample} - OD_{control}$

V_1: The volume of the sample, 0.02 mL

V_2: The volume of the extraction solution, 1 mL

T: Reaction time, 30 min

m: The weight of the sample, g

10^3: 1 mg = 10^3 μg

f: Dilution factor of sample before test

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three sweet potato 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)	80.00	160.00	240.00
%CV	3.8	3.2	2.6

Inter-assay Precision

Three sweet potato 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)	80.00	160.00	240.00
%CV	6.4	5.2	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 100.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	80.00	160.00	240.00
Observed Conc. (m)	79.50	156.00	250.00
Recovery rate (%)	99	98	104

Sensitivity

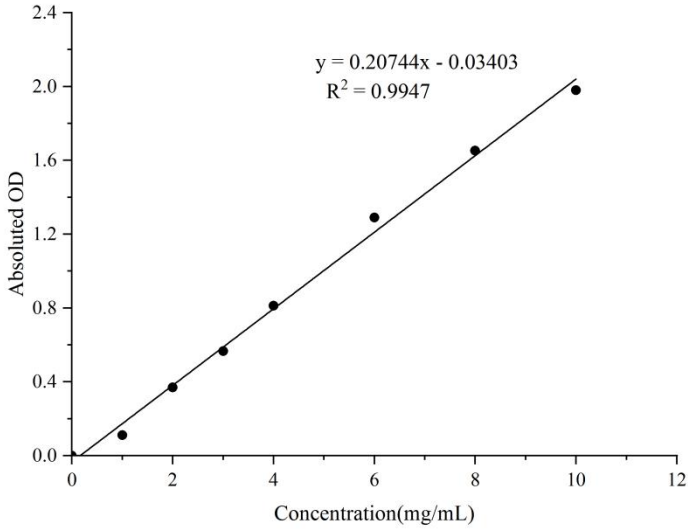
The analytical sensitivity of the assay is 6.22 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the

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 (mg/mL)	0	1	2	3	4	6	8	10
OD	0.300	0.412	0.685	0.881	1.115	1.588	1.949	2.262
	0.297	0.407	0.651	0.848	1.107	1.590	1.953	2.296
Average OD	0.299	0.409	0.668	0.865	1.111	1.589	1.951	2.279
Absoluted OD	0.000	0.111	0.369	0.566	0.812	1.290	1.652	1.980



Appendix Π Example Analysis

Example analysis：

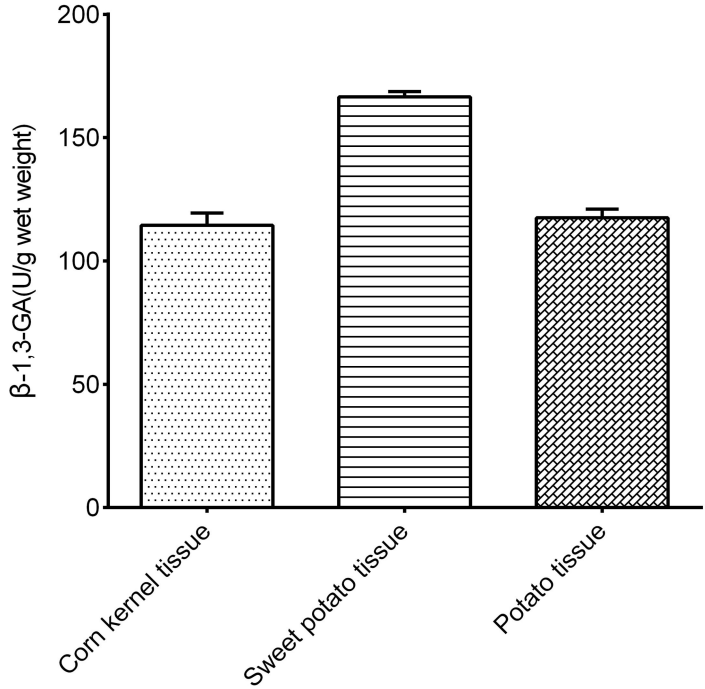
Take 20 μL of sweet potato tissue homogenate and carry the assay according to the operation steps. The results are as follows: standard curve: y=0.20744x - 0.03403, the average OD value of the sample well is 0.650, the average OD value of the control well is 0.474, and the calculation result is:

β-1,3-GA activity (U/g wet weight) = 33.33× (0.650 - 0.474 + 0.03403) ÷ 0.20744 ÷ 0.2

× 1

= 168.73 U/g wet weight

Detect corn kernel tissue, sweet potato tissue and potato tissue, according to the protocol, the result is as follows：


200
150
100
50
0
β-1,3-GA(U/g wet weight)
Corn kernel tissue
Sweet potato tissue
Potato tissue

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.