## (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<sup>®</sup> 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.

# Table of contents

| Assay summary                          | 3  |
|--|----|
| Intended use                           | 4  |
| Detection principle                    | 4  |
| Kit components & storage               | 4  |
| Materials prepared by users            | 5  |
| Reagent preparation                    | 5  |
| The key points of the assay            | 6  |
| Sample preparation                     | 7  |
| Operating steps                        | 8  |
| Calculation                            | 9  |
| Appendix I Performance Characteristics |    |
| Appendix П Example Analysis            |    |
| Statement                              | 13 |



# Intended use

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

## **Detection principle**

 $\beta$ -1, 3-glucanase ( $\beta$ -1,3-GA, EC 3.2.1.39) is mainly found in plants and catalyzes the hydrolysis of  $\beta$ -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  $\beta$ -1, 3-GA to enhance plant resistance to adverse external stimuli. Therefore, the determination of  $\beta$ -1, 3-GA activity is widely used in plant pathology and stress physiology research.

 $\beta$ -1,3-GA hydrolyses the  $\beta$ -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.

| Item      | Component           | Size (96 T)                            | Storage                            |
|-----------|---------------------|--|------------------------------------|
| Reagent 1 | Extraction Solution | $120 \text{ mL} \times 1 \text{ vial}$ | 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,<br>shading light |
| Reagent 5 | Standard            | Powder × 1 vial                        | 2-8°C, 12 months                   |
|           | Microplate          | 96 wells                               | No requirement                     |
|           | Plate Sealer        | 2 pieces                               |                                    |

## Kit components & storage

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°C)

## **Reagents:**

95% alcohol, 80% alcohol

# **Reagent preparation**

- Equilibrate the buffer solution, substrate, chromogenic agent and standard to 25°C 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

- 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:**

- 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°C to remove supernatant and collect the precipitate.
- 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°C 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°C. 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                             | <b>Dilution factor</b> |
|---|------------------------|
| 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°C, 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  $\mu$ L of control samples to the corresponding tubes. Sample tube: Add 20  $\mu$ L of samples to the corresponding tubes.

- (2) Add 20  $\mu$ L of double distilled water to the standard tubes. Add 20  $\mu$ L of substrate working solution to the sample tubes and control tubes.
- ③ Mix fully and incubate at 37°C for 30 min.
- (d) Add 300  $\mu$ L 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°C with running water.
- $\bigcirc$  Add 560 µL of double distilled water to each tube.
- (7) Mix fully and take 200  $\mu$ L 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  $\mu$ g of reducing sugar at 37°C is defined as 1 unit.

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

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

#### [Note]

 $\Delta A: OD_{sample} - OD_{control}$ 

 $V_1$ : The volume of the sample, 0.02 mL

V<sub>2</sub>: 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<br>240.00<br>4.1 |  |
|---------------------|-----|----------|---------------------------|--|
| 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<br>(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 |
| OD                       | 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  $\mu$ L 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:

 $\beta$ -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:



## 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.