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

**Catalog No：E-BC-K756-M**

**Specification：96T(40 samples)**

**Measuring instrument：Microplate reader(500-510 nm)**

**Detection range：0.01-1.00 mg/mL**

# **Elabscience®** Maltose 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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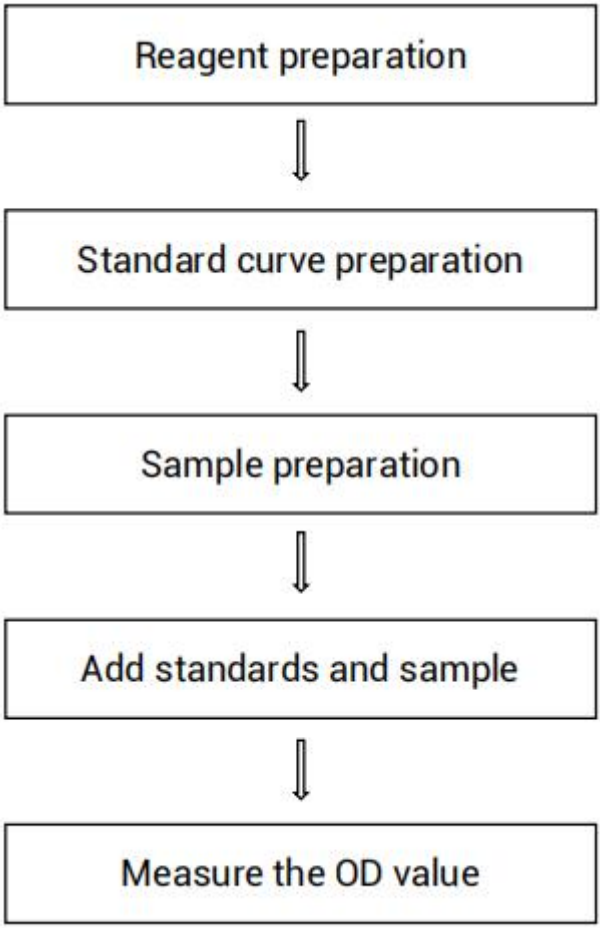
**Assay summary**



## Intended use

This kit can be used to measure maltose content in plant tissue samples.

## Detection principle

Maltose is a disaccharide formed by two molecules of glucose linked by an α-1, 4 glycosidic bond, also known as maltodisaccharide.Maltose is not only involved in the glucose metabolism in the body, but also widely used in the food and pharmaceutical industries, which is one of the important indicators of product quality control.

Maltose is decomposed by enzymes to produce two molecules of glucose. Glucose reacts with the chromogenic agent to form a chromogenic substance, which can show color changes. There is a characteristic absorption peak at 505 nm, and the depth of its color is directly proportional to the content of maltose.

## Kit components & storage

| Item | Component | Size(96T) | Storage |
|---|---|---|---|
| Reagent 1 | Extraction Solution | 60 mL × 1 vial | 2-8℃, 12 months |
| Reagent 2 | Enzyme Regent | Powder × 2 vials | 2-8℃, 12 months, shading light |
| Reagent 3 | Chromogenic Agent A | 10 mL× 1 vial | 2-8℃, 12 months, shading light |
| Reagent 4 | Chromogenic Agent B | 10 mL× 1 vial | 2-8℃, 12 months, shading light |
| Reagent 5 | Standard | Powder × 2 vials | 2-8℃, 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 (500-510 nm, optimum wavelength: 505 nm), Homogenizer, Incubator, Water bath

## Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of enzyme working solution:
Dissolve one vial of enzyme regent with 3 mL of extraction solution, mix well to dissolve. Store at 2-8°C for 7 days protected from light.

③ The preparation of 2 mg/mL standard solution:
Dissolve one vial of standard with 5 mL of extraction solution, mix well to dissolve. Store at 2-8°C for 7 days.

④ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 2 mg/mL standard solution with extraction solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1 mg/mL. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|---|---|---|---|---|---|---|---|---|
| **Concentration (mg/mL)** | **0** | **0.1** | **0.2** | **0.3** | **0.4** | **0.6** | **0.8** | **1** |
| **2 mg/mL Standard (μL)** | 0 | 10 | 20 | 30 | 40 | 60 | 80 | 100 |
| **Extraction solution (μL)** | 200 | 190 | 180 | 170 | 160 | 140 | 120 | 100 |

## Sample preparation

**① Sample preparation**

**Plant tissue sample:**

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

② Homogenize 50 mg tissue in 450 μL extraction solution with a dounce homogenizer at 4℃.

③ Incubate at 95℃ for 5 min. Cool to 25℃ with running water.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. 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 |
|---|---|
| 10% Potato tissue homogenate | 2-5 |
| 10% Chinese cabbage leaf tissue homogenate | 2-5 |
| 10% Sweet potato tissue homogenate | 2-5 |
| 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.

## Operating steps

### Enzymatic reaction

① Standard tube: Add 100 μL of standard solution with different concentrations into 1.5 mL EP tube.
Control tube: Add 100 μL of sample into 1.5 mL EP tube.
Sample tube: Add 100 μL of sample into 1.5 mL EP tube.

② Add 50 μL of extraction solution into control tubes. Add 50 μL of enzyme working solution into standard and sample tubes.

③ Mix fully and incubate at 55°C for 60 min to get enzyme reaction solution. (Cover it tightly to prevent moisture loss)

### Chromogenic reaction

① Take 20 μL of enzyme reaction solution to the corresponding wells

② Add 90 μL of chromogenic agent A and 90 μL of chromogenic agent B to each well.

③ Mix fully with microplate reader and incubate at 37°C for 30 min. Measure the OD value of each well at 505 nm, as A. $\Delta A = A_{sample} - A_{control}$.

## 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**:

**Plant tissue sample:**

$$\text{maltose content (mg/g wet weight)} = \frac{\Delta A - b}{a} \div \frac{m}{V} \times f$$

**[Note]**

$\Delta A = A_{sample} - A_{control}$.

m: The wet weight of sample, g.

V: The volume of extraction solution in the sample preparation step, mL.

f: Dilution factor of sample before tested.

# Appendix I Performance Characteristics

**1. Parameter:**

**Intra-assay Precision**

Three potato tissue 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 (mg/mL) | 0.30 | 0.50 | 0.70 |
| %CV | 4.5 | 3.5 | 1.5 |

**Inter-assay Precision**

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

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (mg/mL) | 0.30 | 0.50 | 0.70 |
| %CV | 5.0 | 2.5 | 3.1 |

**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 101%.

| | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| Expected Conc. (mg/mL) | 0.30 | 0.50 | 0.70 |
| Observed Conc. (mg/mL) | 0.28 | 0.52 | 0.74 |
| Recovery rate (%) | 93 | 104 | 105 |

**Sensitivity**

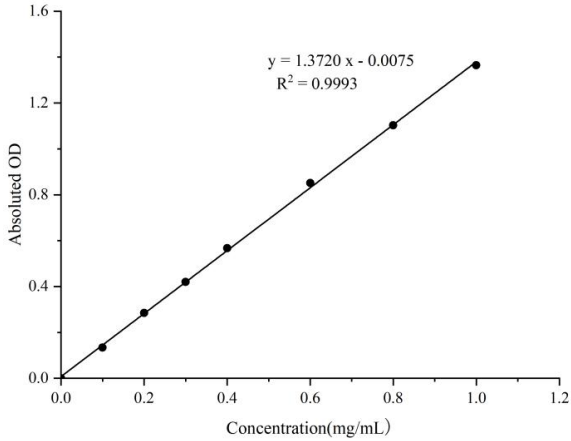
The analytical sensitivity of the assay is 0.01 mg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the

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** | **0.1** | **0.2** | **0.3** | **0.4** | **0.6** | **0.8** | **1** |
|---|---|---|---|---|---|---|---|---|
| **OD value** | 0.054 | 0.189 | 0.340 | 0.477 | 0.622 | 0.922 | 1.139 | 1.402 |
| | 0.062 | 0.195 | 0.346 | 0.480 | 0.628 | 0.897 | 1.183 | 1.444 |
| **Average OD** | 0.058 | 0.192 | 0.343 | 0.478 | 0.625 | 0.910 | 1.161 | 1.423 |
| **Absoluted OD** | 0 | 0.134 | 0.285 | 0.420 | 0.567 | 0.851 | 1.103 | 1.365 |



## Appendix Π Example Analysis

**Example analysis：**

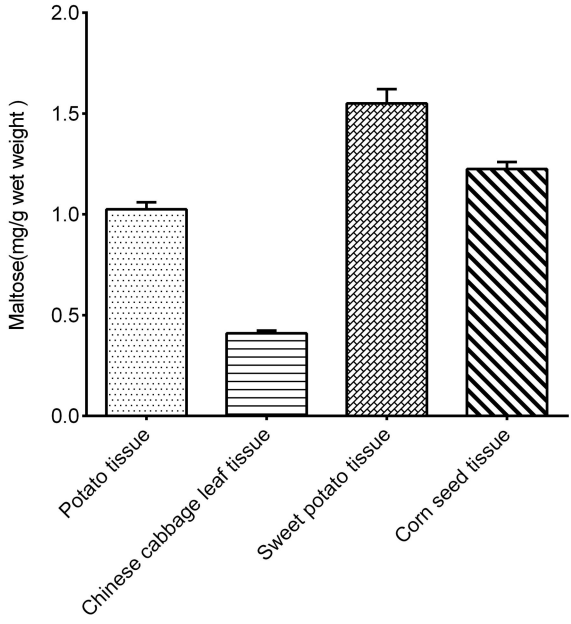
Take 100 μL of 10% sweet potato tissue homogenate which dilute for 2 times and carry the assay according to the operation steps.

Standard curve: y= 1.3720x - 0.0075, the average OD value of the control well is 0.545, the average OD value of the sample well is 0.656, and the calculation result is:

maltose content (mg/g wet weight) = (0.656 - 0.545 + 0.0075) ÷ 1.3720 × (0.9 ÷ 0.1) × 2

= 1.55 mg/g wet weight

Detect 10% potato tissue homogenate (dilute for 2 times), 10% chinese cabbage leaf tissue homogenate (dilute for 2 times), 10% sweet potato tissue homogenate (dilute for 2 times), 10% corn seed tissue homogenate (dilute for 2 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.