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

Catalog No：E-BC-K754-M

Specification：48T(22 samples)/96T(46 samples)

Measuring instrument：Microplate reader(500-520 nm)

Detection range：0.007-1.00 mg/mL

Elabscience® Trehalose Colorimetric Assay Kit (Enzyme Method)

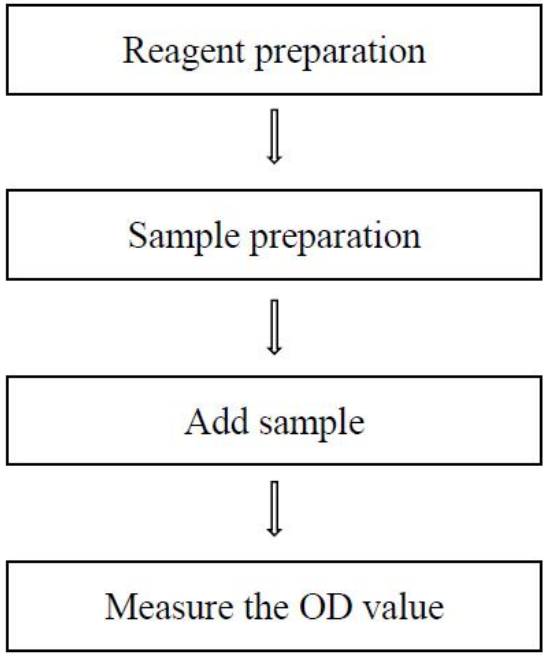
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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



Intended use

This kit can be used to measure the trehalose content in fungus, algae and plant tissue samples.

Detection principle

Trehalose, a non-reducing disaccharide composed of two glucose molecules, is widely found in lower plants, invertebrates and microorganisms. Trehalose is the main energy storage substance for many insects and fungi. In addition, trehalose has a non-specific protective effect on organisms and biological macromolecules. This special property makes it widely used in many fields such as cosmetics, food industry, medicine and agriculture.

This kit specifically hydrolyzes trehalose into two molecules of glucose by trehalose enzyme, and then detects the glucose content by the GOD-POD method. Control well is set up to eliminate the influence of the glucose background value, thereby determining the trehalose content in the sample. It has the characteristics of strong specificity and high sensitivity.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Reagent	0.3 mL × 1 vial	0.6 mL × 1 vial	2-8℃, 12 months shading light
Reagent 2	Chromogenic Agent A	6 mL × 1 vial	12 mL × 1 vial	2-8℃, 12 months shading light
Reagent 3	Chromogenic Agent B	4.5 mL × 1 vial	9 mL × 1 vial	2-8℃, 12 months shading light
Reagent 4	Trehalose Standard	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months shading light
	Microplate	48 wells	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 (500-520 nm, optimum wavelength: 510 nm), Incubator, Water bath

Reagent preparation

① Keep enzyme reagent on ice during use. Equilibrate other reagents to 25°C before use.

② The preparation of 10 mg/mL standard solution:
Dissolve one vial of trehalose standard with 1 mL of double distilled water, mix well to obtain the 10 mg/mL standard solution. Store at 2-8°C for 1 month.

③ The preparation of 1 mg/mL standard solution:
Before testing, please prepare sufficient 1 mg/mL standard solution according to the test wells. For example, prepare 50 μL of 1 mg/mL standard solution (mix well 5 μL of 10 mg/mL standard solution and 45 μL of double distilled water). The 1 mg/mL standard solution should be prepared on spot and used up within 8 hours.

Sample preparation

① Sample preparation

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 0.1 g).

② Add tissue samples into 2 mL EP tube and heated in a 90°C water bath for 10 min.

③ Add 0.9 mL of double distilled water into the tubes and homogenize with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 25℃ 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% Pleurotus djamor pileus tissue homogenate	8-16
10% Pleurotus ostreatus pileus tissue homogenate	5-10
10% Lentinus edodes pileus tissue homogenate	2-4

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

Operating steps

① Sample well: Add 10 μL of sample to sample wells.
Control well: Add 10 μL of sample to control wells.
Standard well: Add 10 μL of 1 mg/mL standard to standard wells.

② Add 10 μL of enzyme reagent to sample and standard wells.

③ Add 100 μL of chromogenic agent A to sample and standard wells. Add 110 μL of chromogenic agent A to control wells. Add 120 μL of chromogenic agent A to blank wells.

④ Add 80 μL of chromogenic agent B to each well.

⑤ Mix fully with microplate reader for 5 s and incubate at 37°C for 30 min protected from light. Measure the OD value of each well at 510 nm, as A.

Calculation

The tissue sample:

$$\text{Trehalose content (mg/g wet weight)} = \frac{A_{sample} - A_{control}}{A_{standard} - A_{blank}} \times c \times f \div \frac{m}{V}$$

[Note]

c: The concentration of standard, 1 mg/mL.

f: Dilution factor of sample before test.

m: The wet weight of sample, g.

V: The volume of double distilled water in the preparation step of tissue, mL.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three pleurotus djamor pileus 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)	0.2	0.5	0.8
%CV	1.3	2.6	1.8

Intra-assay Precision

Three pleurotus djamor pileus 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)	0.2	0.5	0.8
%CV	6.6	3.6	1.9

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. (mg/mL)	0.2	0.5	0.8
Observed Conc. (mg/mL)	0.19	0.50	0.78
Recovery rate (%)	97	100	97

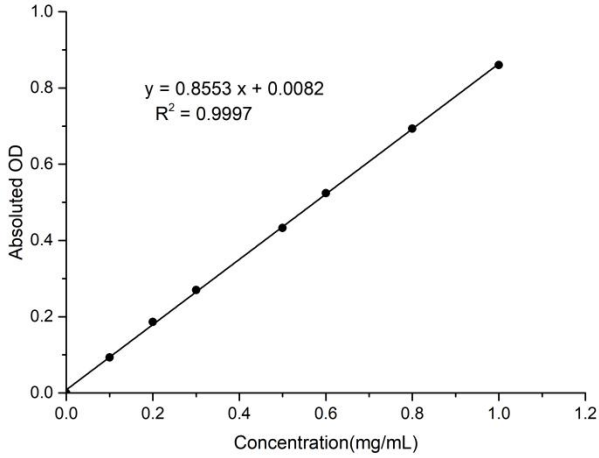
Sensitivity

The analytical sensitivity of the assay is 0.007 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	0.5	0.6	0.8	1.0
OD value	0.054	0.146	0.235	0.321	0.485	0.579	0.742	0.912
	0.055	0.151	0.247	0.329	0.491	0.579	0.753	0.918
Average OD value	0.055	0.148	0.241	0.325	0.488	0.579	0.748	0.915
Absoluted OD value	0.000	0.093	0.186	0.270	0.433	0.524	0.693	0.860



Appendix Π Example Analysis

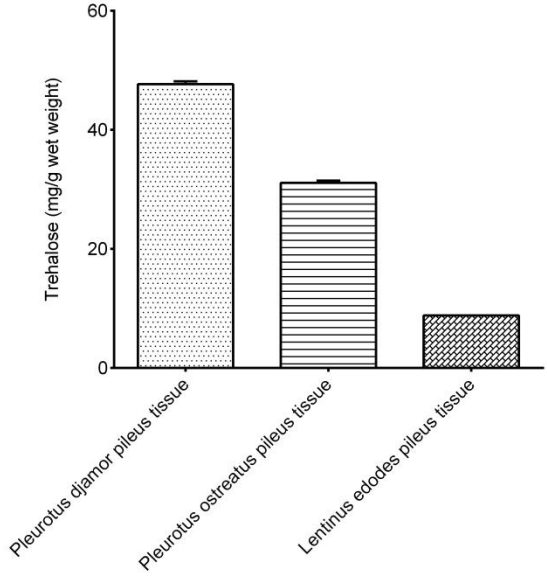
Example analysis：

Take 10 μL of 10% pleurotus ostreatus pileus tissue homogenate which dilute for 6 times and carry the assay according to the operation steps. The results are as follows:

The OD value of the sample well is 0.546, the OD value of the control well is 0.054, the OD value of the standard well is 0.908, the OD value of the blank well is 0.053, and the calculation result is:

Trehalose content (mg/g wet weight) = (0.546 - 0.054) ÷ (0.908 - 0.053) × 1 × 6 ÷ 0.1 × 0.9 = 31.07 mg/g wet weight

Detect 10% pleurotus djamor pileus tissue homogenate (dilute for 8 times), 10% pleurotus ostreatus pileus tissue homogenate (dilute for 6 times) and 10% lentinus edodes pileus 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.