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

**Catalog No：E-BC-K754-M**

**Specification：96T(40 samples)**

**Measuring instrument：Microplate reader（505-515 nm）**

**Detection range：0.05-1.9 mg/mL**

# **Elabscience**® Trehalose 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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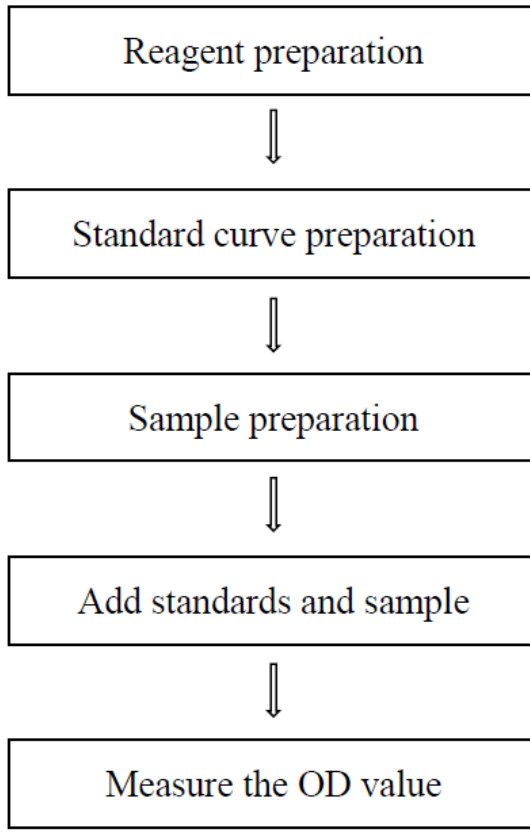
**Assay summary**



## Intended use

This kit can measure trehalose content in serum, plasma, tissue and cell samples.

## Detection principle

Trehalose is found in a large number of organisms, including bacteria, algae, yeast, plants, insects, and other invertebrates. Because trehalose has unique biological characteristics different from other carbohydrates, it can protect the protein, lipid, carbohydrate, nucleic acid and other components of biological cells from damage under the harsh environment such as drought, high temperature, dehydration, freezing, high osmotic pressure and toxic substances.

The determination method was a highly specific enzyme method, trehalase was used to hydrolyze trehalose into 2 molecules of glucose, and then the glucose content was detected by GOD-POD method. Other disaccharides such as maltose and lactose did not interfere with the determination.

## Kit components & storage

| Item | Component | Size (96 T) | Storage |
|---|---|---|---|
| Reagent 1 | Enzyme Reagent | 1 mL ×1 vial | -20ºC, 12 months |
| Reagent 2 | Accelerator | Powder ×1 vial | -20ºC, 12 months |
| Reagent 3 | Chromogenic Agent A | 20 mL ×1 vial | 2-8ºC, 12 months |
| Reagent 4 | Chromogenic Agent B | 16 mL×1 vial | 2-8ºC, 12 months |
| Reagent 5 | Standard | Powder ×1 vial | 2-8ºC, 12 months |
| | Microplate | 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 (505-515 nm, optimum wavelength: 510 nm)，Water bath

## Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of accelerator working solution：
Dissolve one vial of accelerator with 2.2 mL of double distilled water, mix well to dissolve. Aliquoted storage at -20 ℃ for a week, and avoid repeated freeze/thaw cycles is advised.

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. Store at 2-8 ℃ for 14 days protected from light.

④ The preparation of 2 mg/mL standard solution：
Before testing, please prepare sufficient 2 mg/mL standard solution according to the test wells. For example, prepare 1000 μL of 2 mg/mL standard solution (mix well 800 μL of double distilled water and 200 μL of 10 mg/mL standard solution). The standard solution should be prepared on spot and used up within 1 day.

⑤ 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 double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.4, 0.6, 0.8, 1.2, 1.4, 1.6, 2 mg/mL. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|---|---|---|---|---|---|---|---|---|
| **Concentration (mg/mL)** | **0** | **0.4** | **0.6** | **0.8** | **1.0** | **1.5** | **1.8** | **2** |
| **2 mg/mL Standard (μL)** | 0 | 40 | 60 | 80 | 100 | 150 | 180 | 200 |
| **Double distilled water (μL)** | 200 | 160 | 140 | 120 | 100 | 50 | 20 | 0 |

## Sample preparation

**Plasma or serum samples:** Test directly, samples can be stored at -80 ℃ for a month.

**Tissue sample:**

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

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL 80℃ double distilled water with a dounce homogenizer.

④ Centrifuge at 10000×g for 10 minutes at 25℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

**Cell (adherent or suspension) samples:**

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells in cold PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL 80℃ double distilled water with a ultrasonic cell disruptor at 4℃ (Settings: power: 20% or 200 W, 3 s interval for 10 s, repeat 30 times).

④ The samples were extracted by shaking for 30 min at 25℃, then centrifuged at 10000×g for 10 min at 25℃, and collect supernatant and keep it on ice for detection.

**[Note]:** For acidic samples, NaOH (2M) should be used to adjust the pH value to about 7.4, and then stand at room temperature for 30 min, and take supernatant for direct detection.

## The key points of the assay

① If $OD_{sample}$ > 1, the sample can be diluted with double distilled water before testing.

② If $OD_{sample}$ - $OD_{control}$ < 0.005 and $OD_{sample}$ > 1, The volume of sample can be increased (For example, if the volume of the sample increases by 30 μL, the chromogenic agent A decreases by 30 μL), or increase the weight of the sample to be weighed during homogenization, and pay attention to the changed volume or mass into the formula for calculation.

## Operating steps

① Standard well: Add 10 μL of standard solution with different concentrations into the corresponding well.
Sample well: Add 10 μL of sample into sample well.
Control well: Add 10 μL of sample into control well.

② Add 10 μL of enzyme reagent into sample wells.

③ Add 10 μL of accelerator working solution into each well.

④ Add 90 μL of chromogenic agent A into sample wells.

⑤ Add 100 μL of chromogenic agent A into standard and control wells.

⑥ Add 80 μL of chromogenic agent B into each well.

⑦ Mix fully and incubate at 25 ℃ for 30 min protected from light. Measure the OD values of each well at 510 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. Serum and plasma samples:**

$$\text{trehalose content (mg/mL)} = (\Delta A_{510} - b) \div a \div 180.16 \div 2 \times 342.3 \times f$$

**2. Tissue sample:**

$$\text{trehalose content (mg/g wet weight)} = (\Delta A_{510} - b) \div a \div 180.16 \div 2 \times 342.3 \div \frac{m}{V_1} \times f$$

**3. Cell sample:**

$$\text{trehalose content (mg/10^6)} = (\Delta A_{510} - b) \div a \div 180.16 \div 2 \times 342.3 \div \frac{n}{V_2} \times f$$

**[Note]**

$\Delta A_{510}$: $\Delta A_{510} = A_{Sample} - A_{Control}$.

180.16：The molecular weight of glucose.

2：1 trehalose is decomposed into 2 molecules of glucose.

342.3：The molecular weight of trehalose.

f：Dilution factor of sample before test.

m：The wet weight of sample, g.

n：The number of cell sample/10^6.

$V_1$：The volume of buffer solution in the preparation step of tissue, mL.

$V_2$：The volume of buffer solution in the preparation step of cell, mL.

# Appendix I Performance Characteristics

## 1. Parameter:

### Intra-assay Precision

Three mouse plasma 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）** | 0.25 | 1.00 | 1.50 |
| **%CV** | 4.0 | 3.0 | 2.1 |

### Inter-assay Precision

Three mouse plasma were assayed 20 times in duplicate by three operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (mg/mL）** | 0.25 | 1.00 | 1.50 |
| **%CV** | 5.6 | 5.2 | 4.5 |

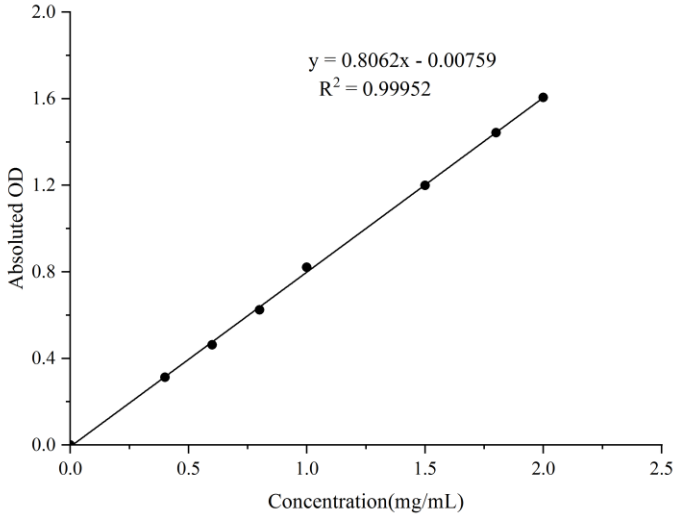
### Sensitivity

The analytical sensitivity of the assay is 0.05 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** | **0.4** | **0.6** | **0.8** | **1.0** | **1.5** | **1.8** | **2** |
|---|---|---|---|---|---|---|---|---|
| **OD Value** | 0.052 | 0.362 | 0.511 | 0.672 | 0.880 | 1.245 | 1.492 | 1.658 |
| | 0.049 | 0.365 | 0.516 | 0.678 | 0.864 | 1.255 | 1.495 | 1.653 |
| **Average OD** | 0.051 | 0.364 | 0.514 | 0.675 | 0.872 | 1.250 | 1.494 | 1.656 |
| **Absoluted OD** | 0.000 | 0.313 | 0.463 | 0.625 | 0.822 | 1.200 | 1.443 | 1.605 |



# Appendix II Example Analysis

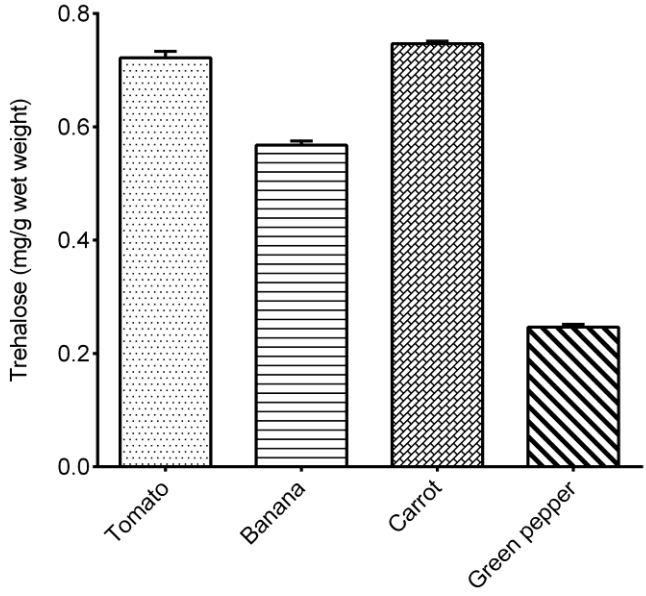
**Example analysis:**

Take 10 μL of 10% tomato tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.8062 x – 0.00759, the OD value of the sample is 0.449, the OD value of the control is 0.396, and the calculation result is:

$$\text{trehalose content (mg/g wet weight)} = (0.449 - 0.396 + 0.00759) \div 0.8062 \div 180.16 \div 2 \times 342.3 \div 0.1 \times 1 = 0.71 \text{ mg/g wet weight}$$

Detect 10% tomato tissue homogenate, 10% banana tissue homogenate, 10% carrot tissue homogenate and 10% green pepper tissue homogenate, 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.