# Trehalose Microplate Assay Kit

# User Manual

**Catalog # CAK1029**

(Version 2.4H)

Detection and Quantification of Trehalose Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

**For research use only. Not for diagnostic or therapeutic procedures.**

Cohesion BIOSCIENCES

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## I. INTRODUCTION

Trehalose is a naturally occurring disaccharide containing two glucose molecules bound in an α,α-1,1 linkage. This structure results in a chemically stable, non-reducing sugar with many important functional characteristics. Trehalose is commonly found in nature, provides a source of energy, and has been shown to be a primary factor in stabilising organisms during times of freezing, drying and heating. Trehalose Microplate Assay Kit provides a simple and direct procedure for measuring trehalose content in a variety of samples. Trehalose is hydrolysed to glucose by trehalase, and the glucose is oxidized by glucose Oxidase, and can be measured at a colorimetric readout at 505 nm.

## II. KIT COMPONENTS

| Component | Volume | Storage |
|---|---|---|
| 96-Well Microplate | 1 plate | |
| Assay Buffer I | 10 ml x 1 | 4 °C |
| Assay Buffer II | Powder x 1 | 4 °C |
| Assay Buffer III | 10 ml x 1 | 4 °C |
| Enzyme I | 30 µl x 1 | 4 °C |
| Enzyme II | Powder x 1 | -20 °C |
| Diluent | 10 ml x 1 | 4 °C |
| Dye Reagent | Powder x 1 | 4 °C |
| Standard | Powder x 1 | 4 °C |
| Plate Adhesive Strips | 3 Strips | |
| Technical Manual | 1 Manual | |

**Note:**

**Assay Buffer II**: add 10 ml Assay Buffer I to dissolve before use.

**Enzyme I**: add 1 ml Diluent to dissolve before use.

**Enzyme II**: add 8 ml Diluent to dissolve before use.

**Dye Reagent**: add 20 ml distilled water to dissolve before use.

**Standard**: add 1 ml distilled water to dissolve before use, then add 0.05 ml into 0.45 ml distilled water, the concentration will be 1 mmol/L.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 505 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

## IV. SAMPLE PREPARATION

1. For liquid samples

Add 0.1 ml sample and 0.1 ml Assay Buffer II to the tube, mix on a vortex mixer, keep at 40 °C for 30 minutes; then add 0.1 ml Assay Buffer III to the tube, a vigorous effervescence should be observed, mix on a vortex mixer.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml distilled water, centrifuged at 12000g for 10 minutes. Add 0.1 ml the supernatant and 0.1 ml Assay Buffer II to a new tube, mix on a vortex mixer, keep at 40 °C for 30 minutes; then add 0.1 ml Assay Buffer III to the tube, a vigorous effervescence should be observed, mix on a vortex mixer.

## V. ASSAY PROCEDURE

Add following reagents in the microplate:

| Reagent | Sample | Control | Standard | Blank |
|---|---|---|---|---|
| Sample | 50 µl | 50 µl | -- | -- |
| Standard | -- | -- | 50 µl | -- |
| Distilled water | -- | 10 µl | -- | 50 µl |
| Enzyme I | 10 µl | -- | 10 µl | 10 µl |
| Enzyme II | 40 µl | 40 µl | 40 µl | 40 µl |
| Dye Reagent | 100 µl | 100 µl | 100 µl | 100 µl |
| Mix, put the plate into the convection oven, 37 °C for 30 minutes, record absorbance measured at 505 nm. | | | | |

**Note:**

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

## VI. CALCULATION

1. According to the volume of sample

$$\text{Trehalose (mmol/L)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} \times n$$

$$= 3 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$\text{Trehalose (mg/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) \times n$$

$$= 3 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the concentration of standard, 1 mmol/L;

W: the weight of sample, g;

$V_{Assay}$: the volume of distilled water, 1 ml;

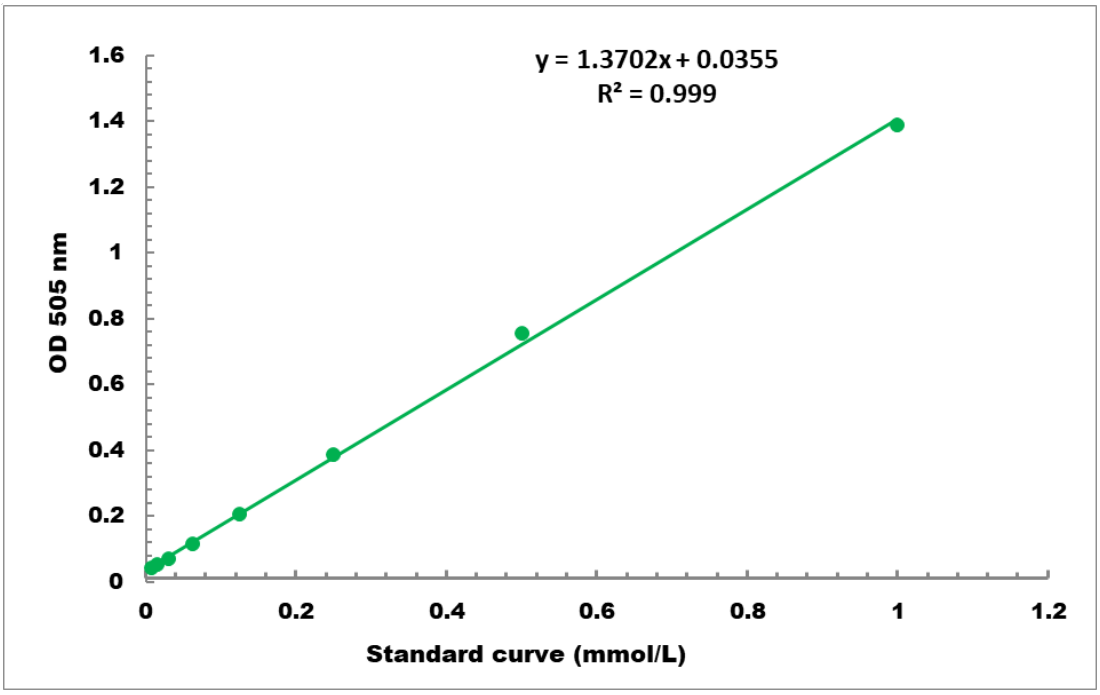
$V_{Standard}$: the volume of standard, 0.05 ml;

$V_{Sample}$: the volume of sample, 0.05 ml;

n: dilution factor, n=3.

## VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.




Detection Range: 0.01 mmol/L - 1 mmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

## IX. NOTES