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.

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	
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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.

3) Reagents must be added step by step, can not be mixed and added together.

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

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

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

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

W: the weight of sample, g;

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

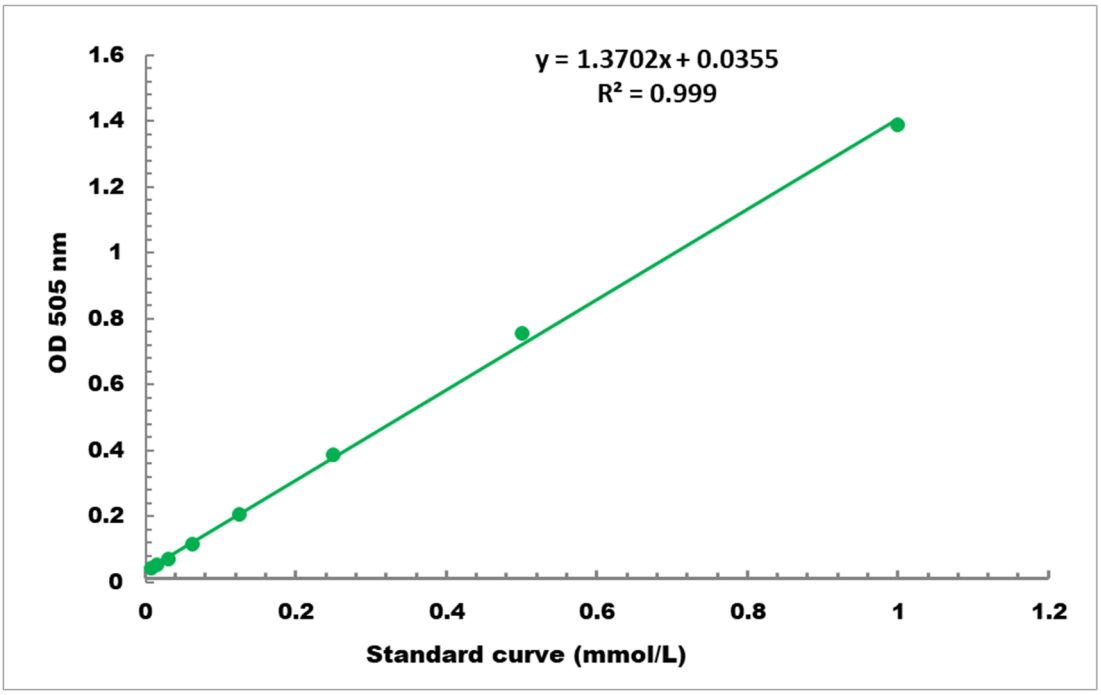
$V_{Standard}$: the volume of standard, 0.05 ml;

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