Trehalose

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1029

(Version 2.5H)

Detection and Quantification of TrehaloseContent 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 Activity Colorimetric 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 BufferI	10 mlx 1	4 °C
Assay BufferII	Powderx 1	4 °C
Assay BufferIII	10 mlx 1	4 °C
Enzyme I	30 µlx 1	4 °C
EnzymeII	Powderx 1	-20 °C
Diluent	10 mlx 1	4 °C
Dye Reagent	Powderx 1	4 °C, keep in dark
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Assay BufferII: Add 10 ml Assay BufferI to dissolve before use.

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 10 mmol/L of standardstock solution, store at 4 °C for 6 monthsafter reconstitution.Then dilute to1 mmol/Lstandard top solutionby adding 50 µl stock solution into 450µl distilled water**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be 1/0.5/0.25/0.125/0.0625/ 0.0312/0.0156 mmol/L.

Enzyme I:Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use.Store at 4 °C for 1-2daysor-20 °C for 1-2 weeks after reconstitution.

Enzyme II: Add 8 ml Diluentto dissolve before use. Keep in dark and store at 4 °C for 2-3 weeks or -20 °C for 2 months.

Dye Reagent: Add 20 ml distilled waterto dissolve before use. Keep in dark and store at 4 °C for 2-3 weeks or -20 °C for 3-4 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For liquid samples

Add 0.1 ml sample and 0.1 mlAssay Buffer IIto the tube, mixon a vortex mixer, keep at 40 °C for 30 minutes; then add0.1 ml Assay Buffer III to the tube, a vigorous effervescenceshould be observed, mixon a vortex mixer.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mldistilled water, centrifuged at 12000gfor 10minutes.Add 0.1 ml the supernatant and 0.1 ml Assay Buffer IIto a new tube, mixon a vortex mixer, keep at 40 °C for 30 minutes; then add0.1 ml Assay Buffer III to the tube, a vigorous effervescenceshould be observed, mixon a vortex mixer.

VI. 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	--	50µl
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 30minutes, record absorbance measured at 505 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Control})-Intercept}{Slope}\times\frac{V_{Standard}}{V_{Sample}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank})\times C_{Protein}\times V_{Sample}}(\mu mol/mg)$$

2.2According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.3According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

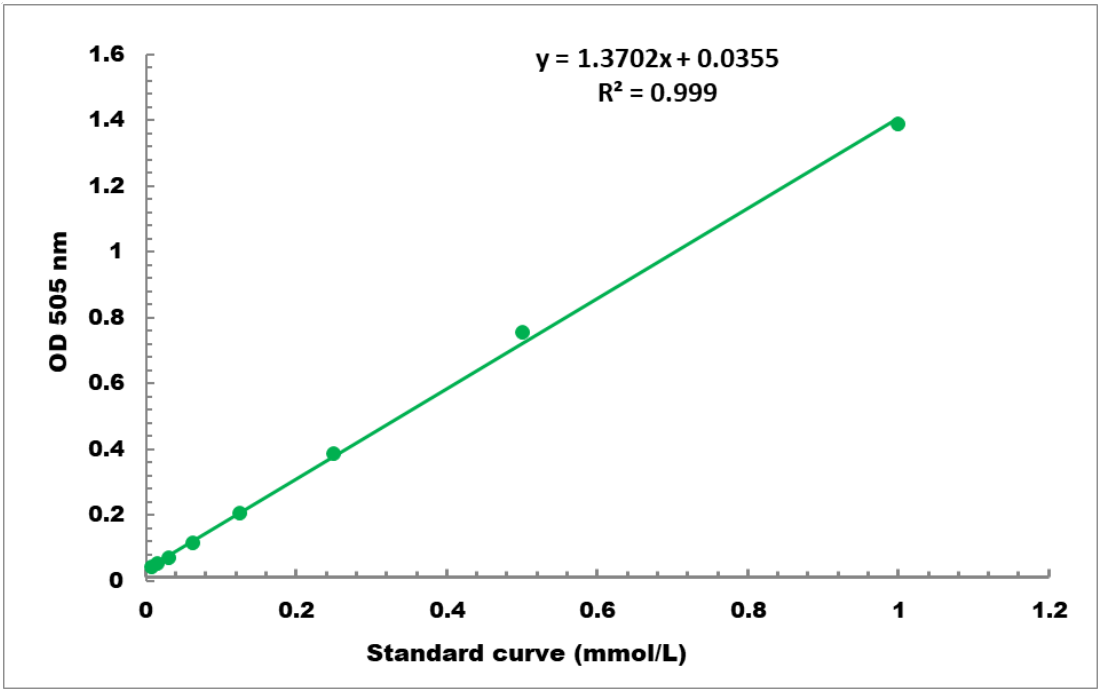
V_{Assay}: the volume of Assay Buffer, μl

$C_{Protein}$: the sample protein concentration, mg/ml

W: the weight of sample, g

VIII. TYPICAL DATA

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



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