Trehalose Microplate Assay Kit

Cat #: orb390735 (manual)

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.

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 stabilizing 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 hydrolyzed to glucose by trehalase, and the glucose is oxidized by glucose Oxidase, and can be measured at a colorimetric readout at 505 nm.

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KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	10 mlx 1	4 °C
Assay Buffer II	Powder x 1	4 °C
Assay Buffer III	10 mlx 1	4 °C
Enzyme I	30 µlx 1	4 °C
Enzyme II	Powder x 1	-20 °C
Diluent	10 mlx 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 1mmol/L.

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

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 add0.1 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 10minutes.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 add0.1 ml Assay Buffer III to the tube, a vigorous effervescence should be observed, mix on a vortex mixer.

ASSAY PROCEDURE

Add following reagents into 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:

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, cannot be mixed and added together.

CALCULATION

1. According to the protein concentration 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, 1mmol/L; W: the weight of sample, g;

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

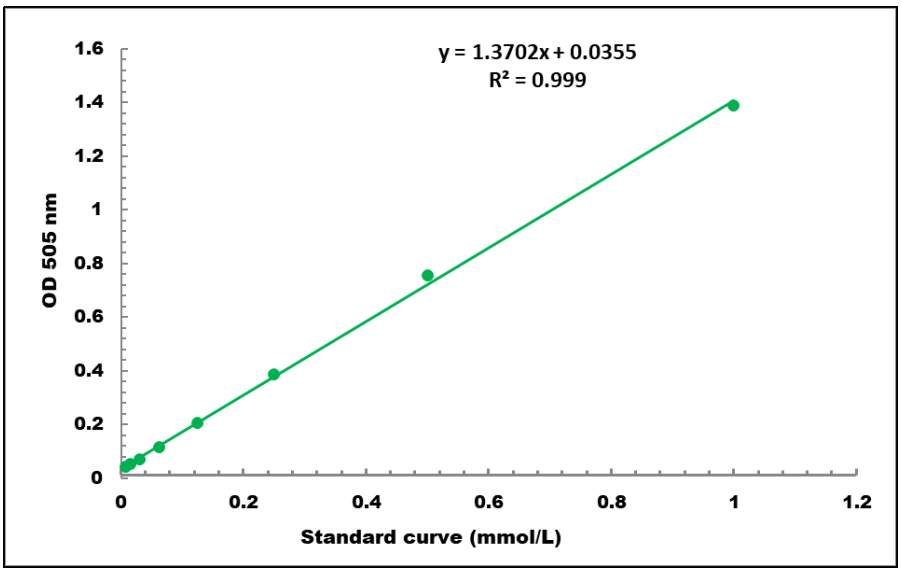
$V_{Standard}$: the volume of standard, 0.05 ml;

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

n: dilution factor, n=3.

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