



# Trehalose Microplate Assay Kit

## User Manual

Catalog # ASK1029

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.**

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**I. INTRODUCTION**

Trehalose is a naturally occurring disaccharide containing two glucose molecules bound in an  $\alpha,\alpha$ -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. The enzyme catalysed reaction products react with 3,5-dinitrosalicylic acid, and can be measured at a colorimetric readout at 540 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	100 µl x 1	4 °C
Dye Reagent	10 ml 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.

**Standard:** add 1 ml distilled water to dissolve before use; then add 0.1 ml into 0.9 ml distilled water, the concentration will be 3 mmol/L.

**III. MATERIALS REQUIRED BUT NOT PROVIDED**

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. 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	Standard	Blank
Sample	50 $\mu$ l	--	--
Standard	--	50 $\mu$ l	--
Distilled water	--	1 $\mu$ l	51 $\mu$ l
Enzyme	1 $\mu$ l	--	--
Mix, put the plate into the oven, 37 °C for 60 minutes.			
Dye Reagent	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
Mix, put the plate into the convection oven, 90 °C for 10 minutes. When cold, record absorbance measured at 540 nm.			



VI. CALCULATION

1. According to the volume of sample

$$\begin{aligned} \text{Trehalose (mmol/L)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &V_{\text{Sample}} / 2 \times 3 \\ &= 4.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

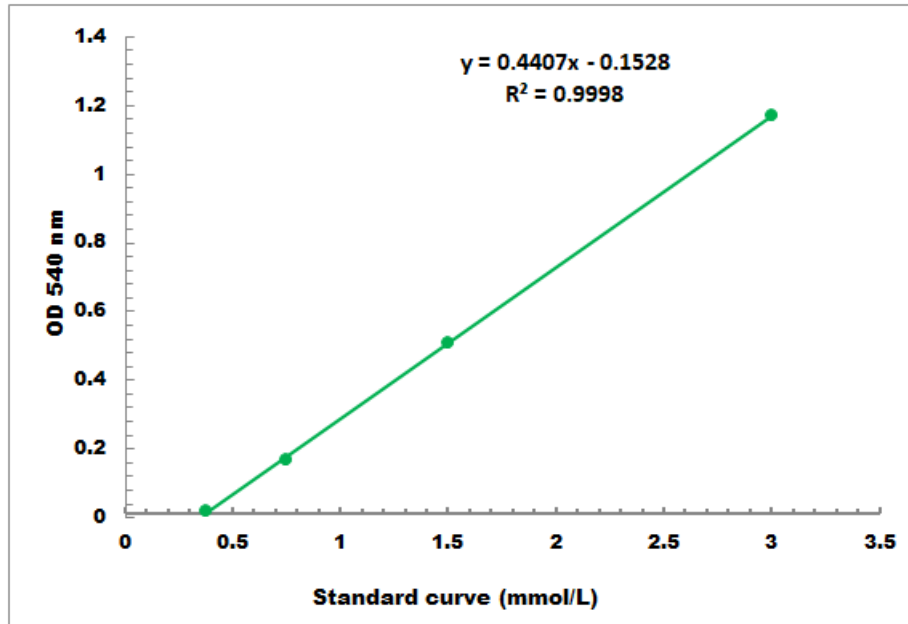
$C_{\text{Standard}}$ : the concentration of Standard, 3 mmol/L;

$V_{\text{Standard}}$ : the volume of sample, 0.05 ml;

$V_{\text{Sample}}$ : the volume of sample, 0.05 ml.

**VII. TYPICAL DATA**

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



Detection Range: 0.3 mmol/L - 3 mmol/L