



Trehalose Microplate Assay Kit

Catalog # AS0029

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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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. 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 BufferI	10 ml x 1	4 °C
Assay BufferII	Powder x 1	4 °C
Assay BufferIII	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 BufferII: add 10 ml Assay BufferI 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 3mmol/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 μ l	--	--
Standard	--	50 μ l	--
Distilled water	--	1 μ l	51 μ l
Enzyme	1 μ l	--	--
Mix, put the plate into the oven,37°C for 60minutes.			
Dye Reagent	100 μ l	100 μ l	100 μ l
Mix, put the plate into the convection oven,90°C for 10minutes. When cold, record absorbance measured at 540 nm.			

VI. CALCULATION

1. According to the volume of sample

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

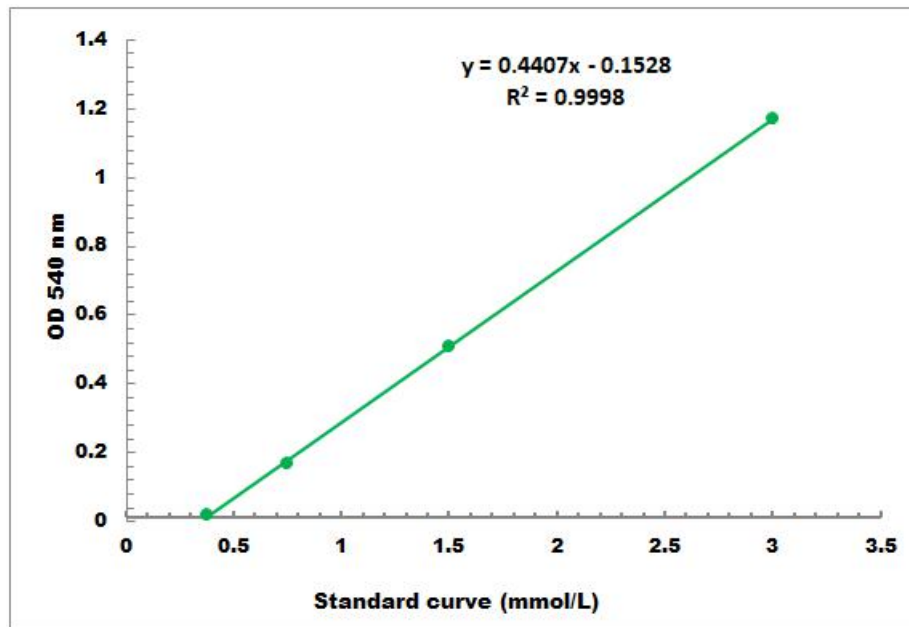
C_{Standard} : the concentration of Standard, 3mmol/L;

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

V_{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: 0.3mmol/L -3mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES