



Trehalase Microplate Assay Kit

User Manual

Catalog # ASK1015

Detection and Quantification of Trehalase (THL) Activity 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

Trehalase is a glycoside hydrolase enzyme that catalyzes the conversion of trehalose to glucose. It is found in most animals. It has been reported that more than 90% of total AT activity in *S. cerevisiae* is extracellular and cleaves extracellular trehalose into glucose in the periplasmic space.

The assay is initiated with the enzymatic hydrolysis of the Trehalose by Trehalase. 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 | 30 ml x 4 | 4 °C |
| Substrate | Powder x 1 | 4 °C |
| Reaction Buffer | 5 ml 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:

Substrate: add 2 ml Assay Buffer before use.

Standard: add 1 ml Distilled water to dissolve before use, the concentration will be 5 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. Ice
7. Centrifuge
8. Timer



9. Convection oven



IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.



V. ASSAY PROCEDURE

Add following reagents into the microplate:

| Reagent | Sample | Standard | Blank |
|---|---------------|-----------------|--------------|
| Sample | 40 µl | -- | -- |
| Substrate | 20 µl | -- | -- |
| Mix, put it in the oven, 37 °C for 10 minutes. | | | |
| Standard | -- | 60 µl | -- |
| Distilled water | -- | -- | 60 µl |
| Reaction Buffer | 40 µl | 40 µl | 40 µl |
| Dye Reagent | 100 µl | 100 µl | 100 µl |
| Put it into the convection oven, 90 °C for 10 minutes, record absorbance measured at 540nm. | | | |

VI. CALCULATION

Unit Definition: One unit of Trehalase activity is the enzyme that generates 1 μmol of reducing sugars per minute.

1. According to the protein concentration of sample

$$\begin{aligned}\text{Trehalase (U/mg)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 0.75 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{Trehalase (U/g)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 0.75 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

3. According to the quantity of cell or bacteria

$$\begin{aligned}\text{Trehalase (U}/10^4) &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 0.75 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N\end{aligned}$$

4. According to the volume of sample

$$\begin{aligned}\text{Trehalase (U/ml)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad V_{\text{Sample}} / T \\ &= 0.75 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

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

C_{Standard} : the concentration of Standard, 5 mmol/L = 5 $\mu\text{mol/ml}$;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Sample} : the volume of sample, 0.04 ml;

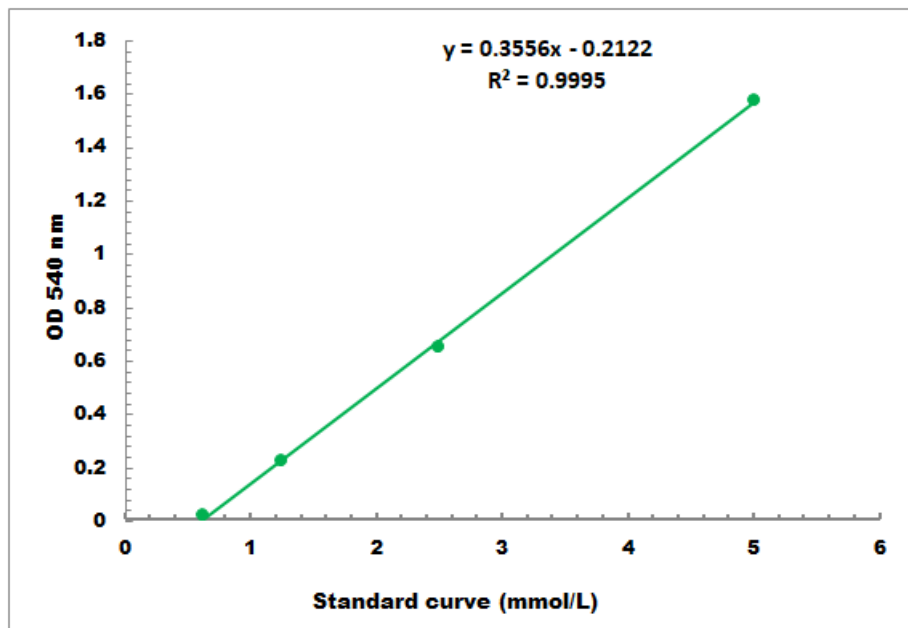
V_{Standard} : the volume of sample, 0.06 ml;

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

T: the reaction time, 10 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.5 mmol/L - 5 mmol/L