

Trehalase Microplate Assay Kit

Cat #: orb219871 (manual)

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.

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.

Trehalase Microplate Assay Kit provides a simple and direct procedure for measuring trehalase activity in a variety of samples. This 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.

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
Positive Control	1 µl 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, mix; then add 0.5 ml into 0.5 ml distilled water, the concentration will be 5 mmol/L.

Positive Control: add 1 ml assay buffer to dissolve before use. Store at -80 °C for 1 month.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer
9. Convection oven

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%, sonicate 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.

ASSAY PROCEDURE

Add following reagents into the microplate:

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

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

CALCULATION

Unit Definition: One unit of trehalase activity is defined as the enzyme generates 1 μmol of reducing sugars per minute.

1. According to the protein concentration of sample

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

2. According to the weight of sample

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

3. According to the quantity of cell or bacteria

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

4. According to the volume of sample

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

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;

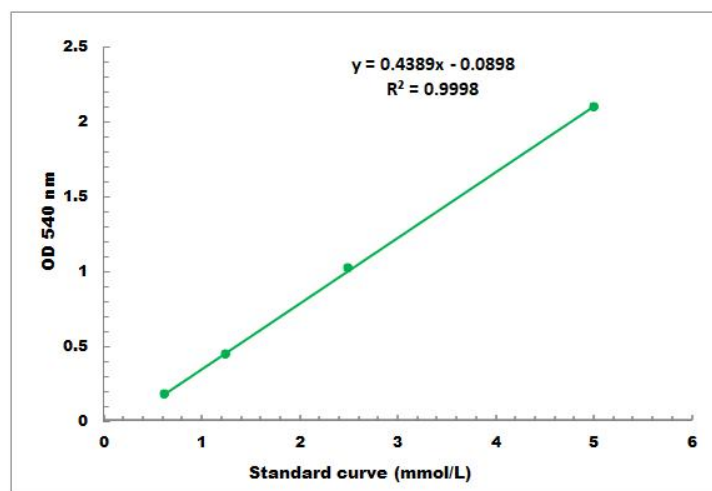
V_{Standard} : the volume of standard, 0.06 ml;

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

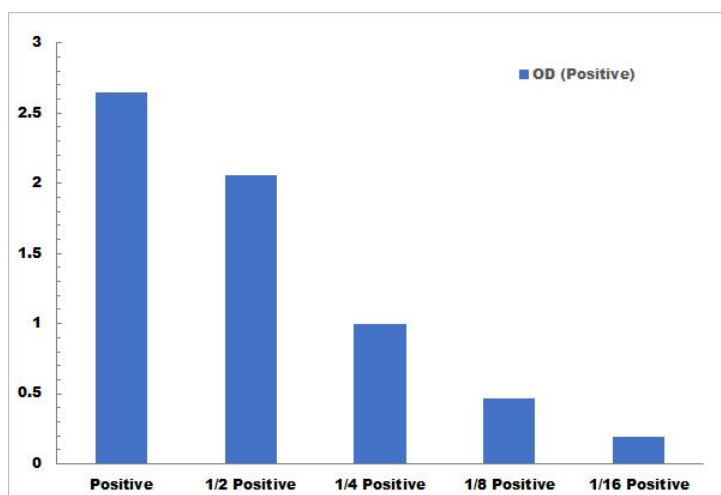
T: the reaction time, 10 minutes.

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



Positive Control reaction in 96-well plate assay with decreasing the concentration