Trehalase Microplate Assay Kit

User Manual

Catalog # CAK1015

(Version 1.4F)

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.

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.

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, and the hydrolyzed product glucose is oxidized by glucose oxidase, can be measured at a colorimetric readout at 505 nm.

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II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
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Note:

Substrate: add 7 ml Reaction Buffer before use. Store at -20 °C for 1 month.

Standard: add 1 ml distilled water to dissolve before use, mix, the concentration will be 50 mmol/L. Store at -20 °C for 1 month.

Enzyme: add 1 ml Reaction Buffer to dissolve before use. Store at -80 °C for 1 month.

Dye Reagent: add 10 ml distilled water to dissolve before use. Store at -20 °C for 1 month.

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

III. 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. Ice
7. Centrifuge
8. Timer

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%, 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.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Sample	20 µl	--	--	--	--
Positive Control	--	--	--	--	20 µl
Substrate	70 µl	70 µl	--	--	70 µl
Standard	--	--	20 µl	--	--
Distilled water	--	20 µl	--	20 µl	--
Reaction Buffer	--	--	70 µl	70 µl	--
Enzyme	10 µl	10 µl	10 µl	10 µl	10 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, put the plate into the convection oven, 37 °C for 5 minutes, record absorbance measured at 505 nm.					

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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of trehalase activity is defined as the enzyme release 2 μmoles of D-glucose per minute.

1. According to the protein concentration of sample

$$\text{Trehalase (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T / 2$$

$$= 5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Trehalase (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T / 2$$

$$= 5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$\text{Trehalase (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T / 2$$

$$= 5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$\text{Trehalase (U/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T / 2$$

$$= 5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the concentration of standard, 50 mmol/L = 50 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

V_{Sample}: the volume of sample, 0.02 ml;

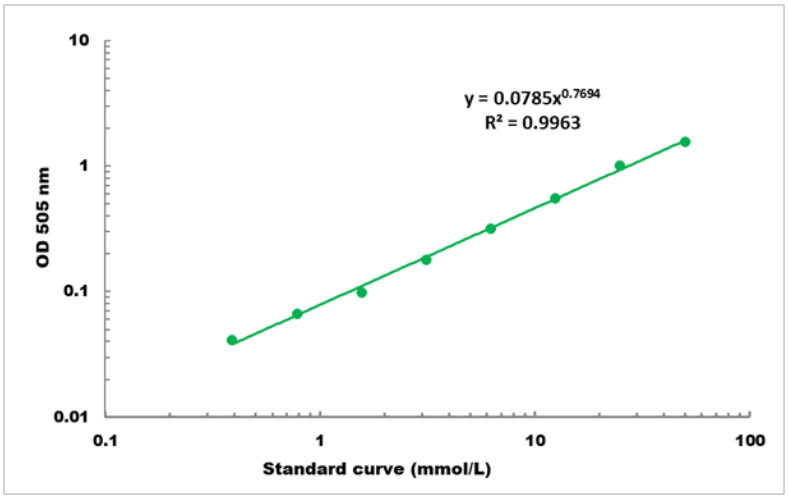
$V_{Standard}$: the volume of standard, 0.02 ml;

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

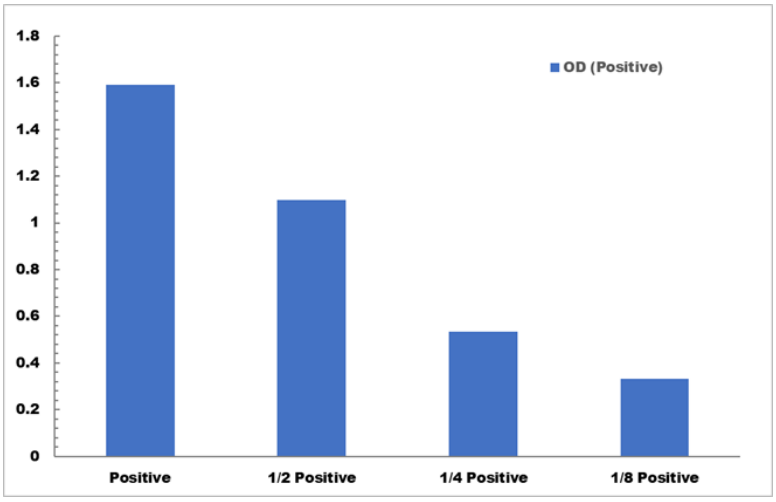
T: the reaction time, 5 minutes.

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 - 50 mmol/L



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES