Trehalase Assay Kit

Cat #: orb219871 (manual)

For Research Use Only. Not For Use in Diagnostic Procedures!

Detection and Quantification of Trehalase (THL) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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 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.

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
Technical Manual	1 Manual	

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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

REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1ml distilled water to generate 50 mmol/L of standard stock solution. Store at -20 °C for 1 month. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 50 mmol/L, 25 mmol/L, 12.5 mmol/L, 6.25 mmol/L, 3.12 mmol/L, 1.56 mmol/L, 0.78mmol/L.

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

Enzyme: Briefly centrifuge prior to opening. 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. Keep in dark and store at -20 °C for 1 month.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use. Store at -80 °C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles

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 8,000g 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 8,000g 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**	Control	Standard	Blank	Positive Control
Sample	20 µl	--	--	--	--
Positive Control	--	--	--	--	20 µl
Substrate	70µl	70µl	--	--	70µl
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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

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 slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T \times 2} \times n\ (U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2 According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Control}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}} \times T \times 2} \quad \text{(U/mL)}$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

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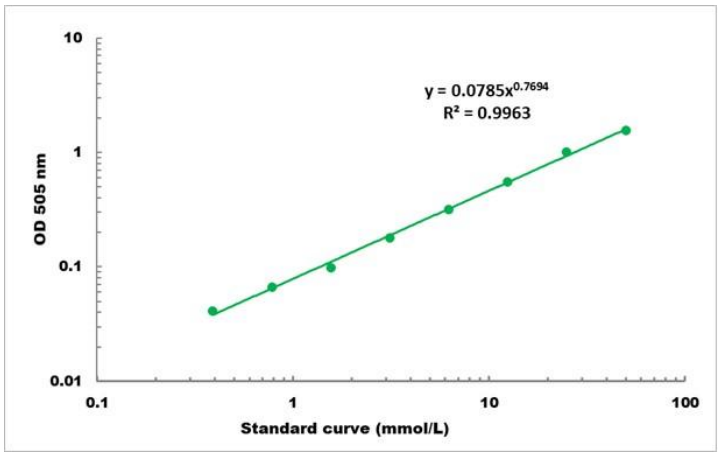
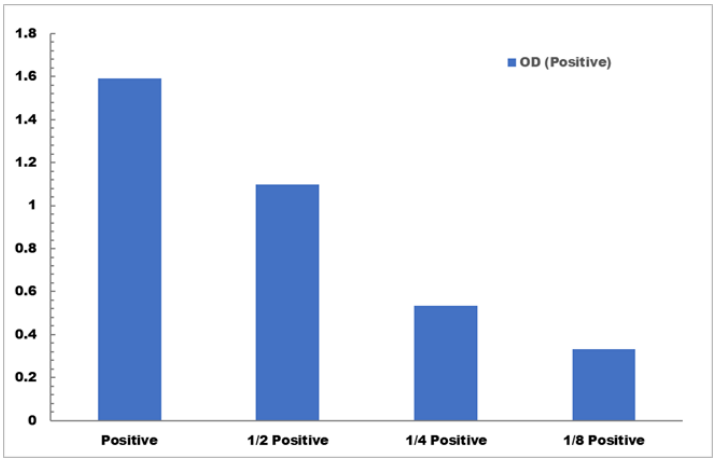
TYPICAL DATA

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



Detection Range: 0.5mmol/L -50mmol/L



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

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