Trehalase Assay Kit

Trehalase Assay Kit is a detection kit for the quantification of Trehalase Content in urine, serum, plasma, tissue extracts, cell lysate and cell culture supernatants.

Catalog number: ARG82017

Package: 96 wells

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INTRODUCTION

The enzyme Trehalase is a glycoside hydrolase, produced by cells in the brush border of the small intestine, which catalyzes the conversion of trehalose to glucose. It is found in most animals.

The non-reducing disaccharide trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is one of the most important storage carbohydrates, and is produced by almost all forms of life except mammals. The disaccharide is hydrolyzed into two molecules of glucose by the enzyme trehalase. There are two types of trehalases found in Saccharomyces cerevisiae, viz. neutral trehalase (NT) and acid trehalase (AT) classified according to their pH optima. NT has an optimum pH of 7.0, while that of AT is 4.5.

Recently 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.

One molecule of trehalose is hydrolyzed to two molecules of glucose by the enzyme trehalase. Enzymatic hydrolysis of trehalose was first observed in Aspergillus niger by Bourquelot in 1893. Fischer reported this reaction in S. cerevisiae in 1895. Since then the trehalose hydrolyzing enzyme, trehalase (α, α-trehalose-1-C-glucohydrolase, EC 3.2.1.28) has been reported from many other organisms including plants and animals. Though trehalose is not known to be produced by mammals, trehalase enzyme is found to be present in the kidney brush border membrane and the intestinal villi membranes. In the intestine the function of this enzyme is to hydrolyze ingested trehalose. Individuals with a defect in their intestinal trehalase have diarrhea when they

eat foods with high trehalose content, such as mushrooms. Trehalose hydrolysis by trehalase enzyme is an important physiological process for various organisms, such as fungal spore germination, insect flight, and the resumption of growth in resting cells. [Wikipedia Trehalase]

PRINCIPLE OF THE ASSAY

The Trehalase Assay Kit is used for determining Trehalase activity in urine, serum, plasma, tissue extracts, cell lysate and cell culture supernatants samples. The assay is initiated with the enzymatic hydrolysis of the Trehalose by Trehalase to reducing sugar. The reaction reducing sugar react with 3,5-dinitrosalicylic acid (DNSA), and can be measured at a colorimetric readout at 540 nm. The Trehalase activity in the sample is then determined by comparing the O.D. of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Microplate	1 X 96-well plate	4°C
Standard (10 µmol)	1 vial (Lyophilized)	4°C
Positive control	1 µl	4°C
Substrate	1 vial (Lyophilized)	4°C
Assay Buffer	4 X 30 ml (ready to use)	4°C
Reaction Buffer	5 ml (ready to use)	4°C
Reaction Dye	10 ml	4°C, protect from light
Plate sealer	3 strips	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Convection oven (37°C, 90°C)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Reaction Dye should be store at 4°C and protect from light.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Collect serum and assay immediately or aliquot and store samples at -20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or below for up to 1 month.

Avoid repeated freeze-thaw cycles.

Urine- Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 8,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at-20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C up to 1 month. Avoid repeated freeze-thaw cycles.

Cell lysate- Collect cell in a centrifuge tube, wash 1-2X by PBS. Discard the supernatant after centrifugation, add 1 ml of Assay buffer pre 5×10^6 cell in the tube. And then sonicate samples (set with power 20%, sonicate for 3 sec. and interval for 10 sec., repeat 30 times). Centrifuge samples 8,000 X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and store samples at -20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

Tissue lysate- Weigh out 0.1 g of tissue, homogenize with 1 ml Assay buffer on ice. Centrifuge samples 8,000 X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and store samples at -20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Standard:** Reconstitute the Standard with 1 ml of distilled water to yield a stock concentration of 10 µmol/ml. Allow the Standard to sit for few minutes with gentle agitation to make sure the Standard is dissolved completely before use. The reconstituted standard stock can be stored at 4°C for up to 1 month. Dilute the stock standard 1:1 with distilled water to yield a working concentration of 5 µmol/ml before use. (ex: 500 µl of stock standard into 500 µl of distilled water)
- **Substrate:** Reconstitute the Substrate with 2 ml of Assay Buffer. Allow the Substrate to sit for few minutes with gentle agitation to make sure the Substrate is dissolved completely before use. The reconstituted Substrate stock can be stored at 4°C for up to 1 month.
- **Positive control:** Prior to using, add 1 ml of Assay Buffer into the Positive control, make sure the Positive control is mixed completely before use. The Positive control can be stored at -80°C for up to 1 month.
- **Sample:** The endogenously reducing sugars in samples will interference the result. If a sample is known to contain reducing sugars, a Sample blank assay can be added to eliminate the endogenous reducing sugars signal. Please refer the assay procedure for the detail.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use.

1. Add **40 µl** per well of **samples**, **positive control** into the appropriate wells in the plate.
   (Optional: for the endogenously reducing sugars containing samples, add 40 µl of each sample in two separate wells of the plate. Well 1 for assay sample and well 2 for sample blank)
2. Add **20 µl** per well of reconstituted **Substrate** into the assay sample and positive control wells. (For sample blank well add **20 µl** of **Assay buffer** per well)
3. Gently tap the plate to ensure **thorough mixing**.
4. Cover the plate with plate sealer and incubate the plate at **37°C for 10 min**.
5. Add **60 µl** of reconstituted **Standard** into the Standard well in the plate.
6. Add **60 µl** of **distilled water** into the blank well in the plate.
7. Add **40 µl** of **Reaction Buffer** per well into all wells.
8. Add **100 µl** of **Reaction Dye** per well into all wells.
9. Gently tap the plate to ensure **thorough mixing**.
10. Cover the plate with plate sealer and incubate the plate at **90°C for 10 min**.
11. Read the OD with a microplate reader at **540 nm** immediately.

Summary of Trehalase Assay Procedure

Reagent	Assay Sample	Sample blank	Positive control	Standard	Blank
Sample	40 µl	40 µl	-	-	-
Positive control	-	-	40 µl	-	-
Substrate	20 µl	-	20 µl	-	-
Assay Buffer	-	20 µl	-	-	-
Mix thoroughly, cover the plate and incubate the plate at 37°C for 10 min.					
Standard	-	-	-	60 µl	-
Distilled water	-	-	-	-	60 µl
Reaction Buffer	40 µl	40 µl	40 µl	40 µl	40 µl
Reaction Dye	100 µl	100 µl	100 µl	100 µl	100 µl
Mix thoroughly, cover the plate and incubate the plate at 90°C for 10 min.					
Read the OD with a microplate reader at 540 nm immediately.					

CALCULATION OF RESULTS

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

2. Calculate the average absorbance values for each set of samples, standards and blank.

3. Calculation:

A. Definition:

OD_{Sample}: OD value from Assay Sample well.

$OD_{Sample\ Blank}$: OD value from Sample Blank well.

$OD_{Standard}$: OD value from Standard well.

OD_{Blank}: OD value from Blank well.

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

$C_{Standard}$: the protein concentration, 5 µmol/ml.

W: the weight of sample, g;

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

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

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

V_{total}: the total volume of sample in Assay buffer, 1 ml. (tissue or cell lysate)

T: the reaction time, 10 minutes.

B. Formula:

a). According to the protein concentration of sample

Trehalase (U/mg) = $[(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) X (V_{Sample} \times C_{Protein}) X T]$

$= 0.75 \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) X C_{Protein}]$

b). According to the weight of sample

Trehalase (U/g) = $[(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) X (W \times V_{Sample} / V_{total}) X T]$

$= 0.75 \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) X W]$

c). According to the quantity of cells

Trehalase ($U/10^4$ cell) = $[(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) X (N \times V_{Sample} / V_{total}) X T]$

$= 0.75 \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) X N]$

d). According to the volume of serum, plasma

Trehalase (μg/ml) = $[(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) X V_{Sample} X T]$

$= 0.75 X (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

Note: *for the Sample blank is assayed, replace* $(OD_{Sample} - OD_{Blank})$ *in above formula with* $(OD_{Sample} - OD_{Sample\ Blank})$.

3. Detection range:

The detection range is from 0.5 mmol/L - 5 mmol/L.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Please note this data is for demonstration only and this kit does not need serial diluted standard.