Trehalose Assay Kit

Catalog No.: abx097995

Size: 100 Assays

Storage: Store the Enzyme 2 vial at -20°C and all other kit components at 4°C.

Application: For quantitative detection of Trehalose concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

Detection Range: 0.01 mmol/L – 1 mmol/L

Introduction: Trehalose is a naturally occurring disaccharide containing two glucose molecules bound in an α,α-1,1 linkage. This structure results in a chemically stable, non-reducing sugar with many important functional characteristics. Trehalose is commonly found in nature, provides a source of energy, and has been shown to be a primary factor in stabilizing organisms during times of freezing, drying and heating. Trehalose is hydrolyzed to glucose by trehalase, and glucose is oxidized by glucose oxidase. The concentration can be measured by measuring the absorbance at 505 nm.

Kit components

1. 96 well microplate
2. Assay Buffer 1: 10 ml
3. Assay Buffer 2: 1 vial
4. Assay Buffer 3: 10 ml
5. Enzyme 1: 30 µl
6. Enzyme 2: 1 vial
7. Diluent: 10 ml
8. Dye Reagent: 1 vial
9. Standard: 1 vial
10. Plate Sealer: 3

Materials Required But Not Provided

1. Microplate reader (505 nm)
2. High-precision pipette and sterile pipette tips
3. Distilled water
4. Mortar
5. Centrifuge and centrifuge tubes
6. Timer
7. Vortexer
8. Convection oven

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Assay Buffer 2 Solution**
  Add 10 ml of Assay Buffer 1 into the Assay Buffer 2 vial to prepare the Assay Buffer 2 Solution. Ensure that the Assay Buffer 2 has completely dissolved prior to use.
- **Enzyme 1 Working Solution**
  Add 1 ml of Diluent to the Enzyme 1 vial and mix thoroughly to prepare the Enzyme 1 Working Solution. Ensure that the Enzyme 1 has completely dissolved prior to use.
- **Enzyme 2 Working Solution**
  Add 8 ml of Diluent to the Enzyme 2 vial and mix thoroughly to prepare the Enzyme 2 Working Solution. Ensure that the Enzyme 2 has completely dissolved prior to use.
- **Dye Reagent Solution**
  Add 20 ml of distilled water to the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.
- **Standard Solution**
  Add 1 ml of distilled water to the Standard vial and mix thoroughly, ensuring that that the Standard has completely dissolved. Add 0.05 ml of this solution to 0.45 ml of distilled water to prepare a 1 ml Standard Solution with concentration 1 mmol/L.

2. Sample

- **Liquid samples**
  Add 0.1 ml of sample and 0.1 ml of Assay Buffer 2 Solution to a new tube, vortex to mix thoroughly, then incubate at 40°C for 30 minutes. Add 0.1 ml of Assay Buffer 3 to the mixture; a vigorous effervescence should be observed. Vortex to mix thoroughly, then assay immediately.

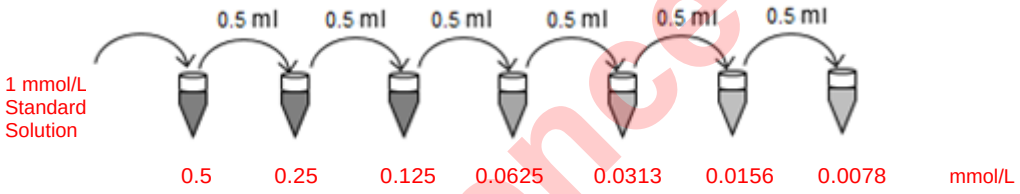
- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of distilled water. Centrifuge at 12,000 × g at room temperature for 10 minutes. Transfer 0.1 ml of the supernatant to a new tube and add 0.1 ml of Assay Buffer 2 Solution. Vortex to mix thoroughly, then incubate at 40°C for 30 minutes. Add 0.1 ml of Assay Buffer 3 to the mixture; a vigorous effervescence should be observed. Vortex to mix thoroughly, then assay immediately.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentrations within the detection range of the kit.

1. Label 7 tubes with 0.5 mmol/L, 0.25 mmol/L, 0.125 mmol/L, 0.0625 mmol/L, 0.0313 mmol/L, 0.0156 mmol/L and 0.0078 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 1 mmol/L Standard Solution to the 1st tube (0.5 mmol/L) and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard, control, and blank wells and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 50 µl of sample to the sample wells and control wells.
4. Add 50 µl of prepared standard solutions to the standard wells.
5. Add 50 µl of distilled water to the blank wells.
6. Add 10 µl of distilled water to the control wells.
7. Add 10 µl of Enzyme 1 Working Solution to the sample wells, standard wells, and blank wells.
8. Add 40 µl of Enzyme 2 Working Solution to all wells.
9. Add 100 µl of Dye Reagent Solution to all wells.
10. Tap the plate gently to mix. Incubate at 37°C for 30 minutes.
11. Read and record absorbance at 505 nm.

C. Calculations

Trehalose concentration per L of sample:

$$\mathbf{Trehalose\ (mmol/L)} = n \times \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = 3 \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Trehalose per g of sample:

$$\mathbf{Trehalose\ (mg/g)} = n \times \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{3}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Control}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Standard}}$	Concentration of highest standard (1 mmol/L)
$\mathbf{W}$	Weight of sample (g)
$\mathbf{V_{Sample}}$	Volume of sample (0.05 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.05 ml)
$\mathbf{V_{Assay}}$	Volume of distilled water used in sample preparation (1 ml)
$\boldsymbol{n}$	Dilution factor (3)