Trehalose

Fluorometric Microplate Assay Kit

User Manual

Catalog # CAK8006

(Version 1.1A)

Detection and Quantification of Trehalose Content 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

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 stabilising organisms during times of freezing, drying and heating. Trehalose Fluorometric Microplate Assay Kit provides a simple and direct procedure for measuring trehalose content in a variety of samples. Trehalose is hydrolysed to glucose by trehalase, and the glucose is oxidized by glucose oxidase, resulting in the generation of an intermediate that reacts with the probe, which can be detected fluorometrically (Ex/Em 535/587).

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Black Microplate	1 plate	
Assay Buffer I	10 ml x 1	4 °C
Assay Buffer II	Powder x 1	4 °C
Assay Buffer III	10 ml x 1	4 °C
Reaction Buffer	20 ml x 2	4 °C
Enzyme I	30 µl x 1	4 °C
Enzyme II	Powder x 2	-20 °C
Probe	Powder x 2	-20 °C, keep in dark
Probe Diluent	1 ml x 2	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Assay Buffer II: add 10 ml Assay Buffer I to dissolve before use. Store at 4 °C. Use within one month.

Enzyme I: add 1 ml Reaction Buffer to dissolve before use. Aliquot & store at -20 °C. Use within one month.

Enzyme II: add 1 ml Reaction Buffer to dissolve before use. Aliquot & store at -20 °C. Use within one month.

Probe: Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent; then add 1 ml Probe Diluent to dissolve. Store at -20 °C, protect from light and moisture. Use within one month.

Standard: add 1 ml distilled water to dissolve before use, then add 0.01 ml into 0.49 ml distilled water, the concentration will be 200 µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For liquid samples

Add 0.1 ml sample and 0.1 ml Assay Buffer II to the tube, mix on a vortex mixer, keep at 40 °C for 30 minutes; then add 0.1 ml Assay Buffer III to the tube, a vigorous effervescence should be observed, mix on a vortex mixer.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml distilled water, centrifuged at 12000g for 10 minutes. Add 0.1 ml the supernatant and 0.1 ml Assay Buffer II to a new tube, mix on a vortex mixer, keep at 40 °C for 30 minutes; then add 0.1 ml Assay Buffer III to the tube, a vigorous effervescence should be observed, mix on a vortex mixer.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Control	Standard	Blank
Reaction Buffer	160 µl	160 µl	160 µl	160 µl
Sample	10 µl	10 µl	--	--
Standard	--	--	10 µl	--
Distilled water	--	10 µl	--	10 µl
Enzyme I	10 µl	--	10 µl	10 µl
Enzyme II	10 µl	10 µl	10 µl	10 µl
Probe	10 µl	10 µl	10 µl	10 µl
Mix, put it in the oven, 37 °C for 5 minutes, protected from light, record fluorescence measured at Ex/Em = 535/587 nm.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

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

VI. CALCULATION

1. According to the volume of sample

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

$$= 0.6 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$\text{Trehalose (mmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) \times n$$

$$= 0.6 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the concentration of standard, 200 μmol/L = 0.2 μmol/ml;

W: the weight of sample, g;

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

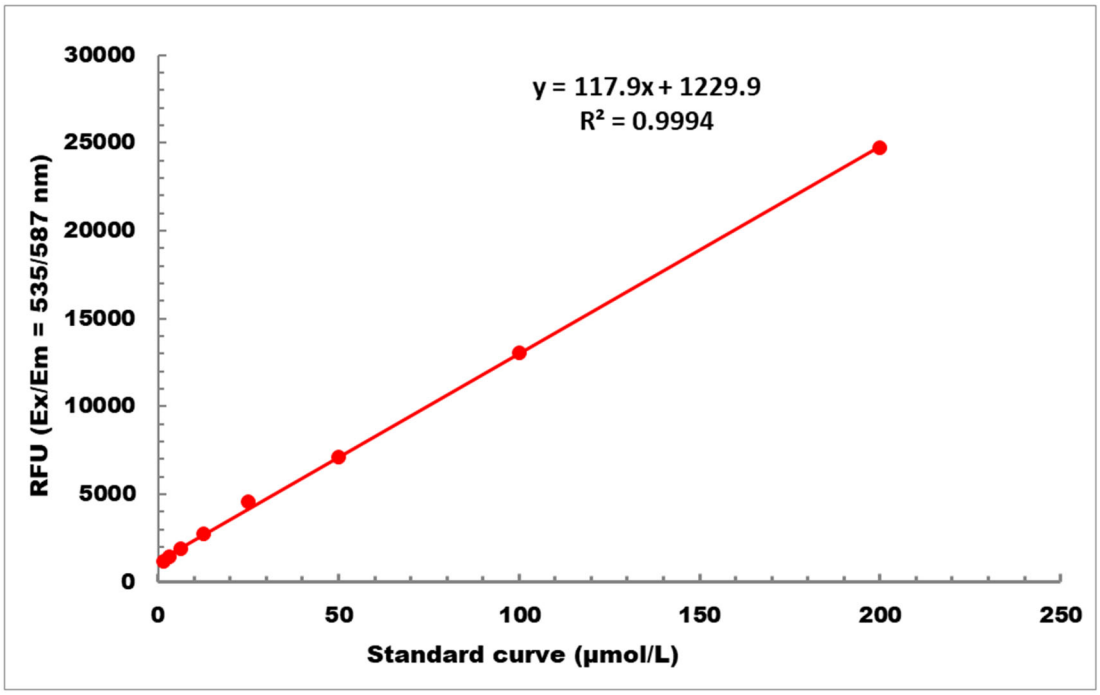
$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml;

n: dilution factor, n=3.

VII. TYPICAL DATA

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



Detection Range: 2 µmol/L - 200 µmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES