Sorbitol Microplate Assay Kit

User Manual

Catalog # CAK1100

(Version 1.2D)

Detection and Quantification of Sorbitol Content in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Sorbitol is one of the 6 carbon sugar alcohols. It is commonly used as an artificial sweetener, as a laxative and in cosmetics as a humectant and thickening agent. Sorbitol is produced naturally in a variety of fruits. It can be produced in humans in small amounts by the reduction of glucose by aldose reductase. Due to its poor ability to diffuse across the cell membrane, sorbitol can be trapped in cells and is believed to be one of the causes of damage (due to osmotic effects) in diabetes. Sorbitol can react with Cu^{2+} under alkaline solution development of intense color with an absorbance at 655 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	2 ml x 1	4 °C
Dye Reagent	2 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Standard: add 1 ml distilled water before use, the concentration is 5 mg/ml.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 655 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml distilled water for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml distilled water, put it in boiling water for 10 minutes, centrifuged at 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For cell culture media and other biological fluids samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Blank	Standard
Sample	160 µl	--	--
Distilled water	--	160 µl	--
Standard	--	--	160 µl
Dye Reagent	20 µl	20 µl	20 µl
Mix well.			
Reaction Buffer	20 µl	20 µl	20 µl
Mix, wait for 30 minutes, centrifuged at 8000g, RT for 10 minutes, take 100 µl supernatant into the microplate, record absorbance measured at 655 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 protein concentration of sample

$$\text{Sorbitol (mg/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

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

2. According to the weight of sample

$$\text{Sorbitol (mg/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

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

3. According to the quantity of cells or bacteria

$$\text{Sorbitol (mg/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

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

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

$C_{Standard}$: the standard concentration, 5 mg/ml;

W: the weight of sample, g;

V_{Sample}: the volume of sample, 0.16 ml;

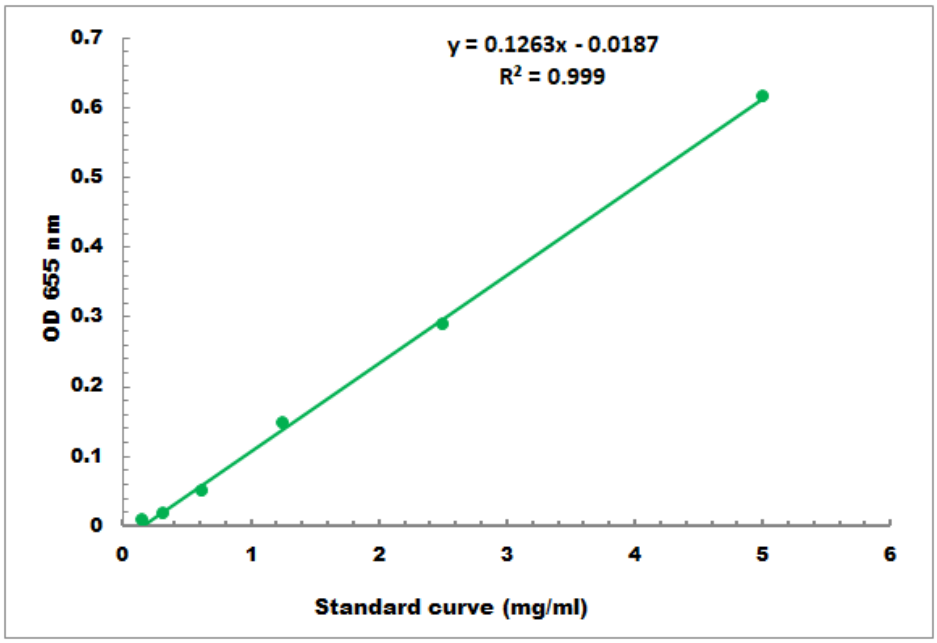
$V_{Standard}$: the volume of standard, 0.16 ml;

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

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

VII. TYPICAL DATA

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



Detection Range: 0.1 mg/ml - 5 mg/ml

VIII. TECHNICAL SUPPORT

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

IX. NOTES