Sorbitol Assay Kit

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

Catalog number: ARG82028

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Sorbitol, less commonly known as glucitol, is a sugar alcohol with a sweet taste which the human body metabolizes slowly. It can be obtained by reduction of glucose, which changes the converted aldehyde group (−CHO) to a primary alcohol group (−CH2OH). Most sorbitol is made from potato starch, but it is also found in nature, for example in apples, pears, peaches, and prunes. It is converted to fructose by sorbitol-6-phosphate 2-dehydrogenase. Sorbitol is an isomer of mannitol, another sugar alcohol; the two differ only in the orientation of the hydroxyl group on carbon 2. While similar, the two sugar alcohols have very different sources in nature, melting points, and uses. [Provide by Wikipedia: Sorbitol]

PRINCIPLE OF THE ASSAY

This Sorbitol Assay Kit is a simple colorimetric assay that measures the amount of Sorbitol present in tissue extracts, cell lysate and cell culture supernatants. Sorbitol can react with Cu^{2+} under alkaline solution development of intense color with an absorbance at O.D. 655 nm. Samples and standards are read with a plate reader. The concentration of sorbitol in the samples is then determined by comparing the O.D. 655 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well Microplate	1 plate	RT
Reaction Buffer	2 mL (ready to use)	4°C
Dye Reagent	2 mL (ready to use)	4°C
Standards (lyophilized)	1 vial	4°C
Plate sealer	3 ea	RT
Technical Manual	1 ea	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 655 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in 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.

Cell and bacteria samples: Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mL of distilled water for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000 x g for 20 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue samples: Weigh out 0.1 g tissue, homogenize with 1 mL of distilled water, and put it in boiling water bath for 10 minutes. Centrifuged at 10,000 x g for 20 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Cell culture medium and other biological fluids: To remove insoluble particles, centrifuge at 10,000 x g for 20 minutes at 4°C. The supernatant should be assayed directly.

REAGENT PREPARATION

- **Standards:** add 1 mL of distilled water to dissolve before use, the concentration will be 5 mg/mL. Use the 5 mg/mL Standards to prepare a series of standards according to the Table below.

Standard tube	Final Standard conc. (mg/mL)	Volume of distilled water (µL)	Volume of 5 mg/mL Standards (µL)
S1	5	0	500
S2	2.5	250	250 of S1
S3	1.25	250	250 of S2
S4	0.625	250	250 of S3
S5	0.313	250	250 of S4
S6	0.156	250	250 of S5

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add **160 µL** of **samples, Blank (distilled water),** or serial **diluted Standards** into 96-well microplate.
2. Add **20 µL** of **Dye Reagent** into each well and mix well.
3. Add **20 µL** of **Reaction Buffer** into each well.
4. Mix well and incubate for **30 minutes** at **room temperature**.
5. Centrifuge at **8,000 x g** for **10 minutes** at **room temperature**.
6. Read the plate with a microplate reader at **O.D. 655 nm**.

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. According to the protein concentration of sample:

Sorbitol (mg/mg)

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

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

6. According to the weight of sample:

Sorbitol (mg/g)

$= [(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} \times W / V_{Assay})$

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

7. According to the quantity of cells or bacteria:

Sorbitol ($mg/10^4$)

$= [(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (N \times V_{Sample} / V_{Assay})$

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

Note:

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

W: the weight of sample, g;

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

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

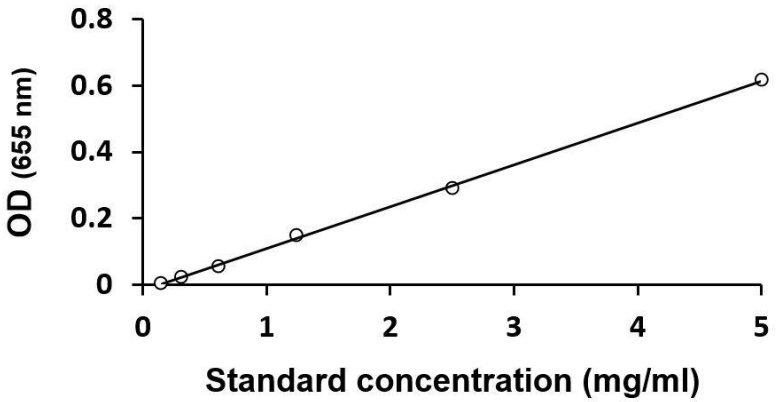
V_{Sample}: the volume of sample, 0.16 mL;

V_{Assay}: the volume of Assay buffer, 1 mL;

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Sorbitol Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.1 mg/mL