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: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 622 1320

Fax: +886 (3) 553 0266

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. It is based on sorbitol dehydrogenase catalyzed oxidation of sorbitol in which the formed NADH reduces a formazan reagent. The intensity of the product color, measured at 450 nm is proportional to the sorbitol concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well Microplate	1 plate	RT
Assay Buffer	30 mL (ready to use)	4°C
Reaction Buffer	10 mL (ready to use)	4°C
Diluent	1 mL (ready to use)	4°C
Enzyme Mix (lyophilized)	1 vial	-20 °C, keep in dark
Dye Reagent A (lyophilized)	1 vial	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
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
- Convection oven

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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. 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 Assay Buffer 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 Assay Buffer, 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: Detect directly.

REAGENT PREPARATION

- **Standards:** Briefly centrifuge prior to opening. Dissolve in **1 ml** distilled water to generate 2**0 µmol/ml** of standard stock solution, store at 4 °C for 2 weeks or -20°C for 6 months after reconstitution. Then dilute to 2 µmol/ml standard top solution by adding **50 µl** stock solution into **450 µl** distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be **2 µmol/ml, 1 µmol/ml, 0.5 µmol/ml, 0.25 µmol/ml, 0.125 µmol/ml, 0.0625 µmol/ml, 0.0312 µmol/ml.**
- **Enzyme Mix**: Briefly centrifuge prior to opening. Add **1 ml** Diluent to dissolve before use. Aliquot & store at-80 °C. Use within 1 month.
- **Dye Reagent A**: Add **9 ml** distilled water to dissolve before use, mix. Keep in dark and store at 4°C for 1 week or-20°C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

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 **80 µl** of **Reaction Buffer** in each well.
2. Add **10 µl** per well of **samples**, **standard** and **Distilled water** into the appropriate well of the 96-well plate.
3. Add **10 µl** of **Enzyme Mix** in each well.
4. Add **90 µl** of **Reaction Dye A** in each well.
5. Add **10 µl** of **Reaction Dye B** in each well.
6. Mix thoroughly, put it into the convection oven, **37°C** for **30 minutes**,
7. Read the absorbance measured at **450 nm** immediately.

Summary of Sorbitol Assay Procedure

Reagent	Sample	Standard	Blank
Reaction Buffer	80 µl	80 µl	80 µl
Sample	10 µl	-	-
Standard	-	10 µl	-
Distilled water	-	-	10 µl
Enzyme Mix	10 µl	10 µl	10 µl
Reaction Dye A	90 µl	10 µl	10 µl
Reaction Dye B	10 µl	10 µl	10 µl
Mix thoroughly, put it into the convection oven, **37°C** for **30 minutes**, Read the absorbance measured at **450 nm** immediately.			

Note: Reagents must be added sequentially and should not be premixed prior to addition.

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Blank and samples.
2. Calculation:

   A. Definition:

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

   W: the weight of sample, g;

   $C_{Standard}$: the concentration of standard, 2 μmol/ml;

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

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

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

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

   B. Formula:

   a).According to the protein concentration of sample:

   Sorbitol (μmol/mg)

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

   b).According to the weight of sample:

   Sorbitol (μmol/g)

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

c).According to the quantity of cells or bacteria:

Sorbitol (μmol/10^4)

= [($C_{Standaard}$ x $V_{Standard}$) x (OD_{Sample} – OD_{Blank}) / ($OD_{Standard}$ – OD_{Blank})] / (N x V_{Sample} / V_{Assay})

d).According to the volume of sample:

Sorbitol (μmol/ml)

= [($C_{Standaard}$ x $V_{Standard}$) x (OD_{Sample} – OD_{Blank}) / [($OD_{Standard}$ – OD_{Blank}) x V_{Sample}]

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.