Technical Bulletin

Sorbitol Assay Kit

Catalogue Number MAK442

Product Description

Sorbitol (glucitol) is a sugar alcohol that is metabolized slowly in the human body. Sorbitol can be obtained from glucose by reducing the aldehyde group to a hydroxyl group. Accumulation of excessive sorbitol in erythrocytes, retinal cells, and Schwann cells has been associated with retinopathy, cataracts, peripheral neuropathy and diabetes. Sorbitol is produced from corn syrup, and found in fruits such as apples, pears, peaches, and prunes. It is widely used as a sugar substitute and as a laxative. It is also utilized in specialty culture media and in healthcare, food and cosmetic products. Sorbitol is measured in biological samples to monitor metabolic pathways and the progression of diabetes.

The sorbitol assay involves an end-point enzymatic reaction coupled with MTT/NAD that results in a colored product with an absorption maximum at 565 nm. The increase in absorbance at 565 nm is directly proportional to the sorbitol concentration. The linear detection range for the assay method is 5 – 1000 µM D-sorbitol for a 20 µL sample.

The Sorbitol Assay Kit is suitable for the quantitative determination of D-sorbitol and evaluation of drug effects on sorbitol metabolism in biological (e.g., blood), food, beverage, and agricultural samples.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer — 10 mL
  Catalog Number MAK442A
- NAD/MTT — 1 mL
  Catalog Number MAK442B
- Enzyme A — 1 vial
  Catalog Number MAK442F
- Enzyme B — 120 µL
  Catalog Number MAK442D
- Standard (50 mM Sorbitol) — 250 µL
  Catalog Number MAK442E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use except for Enzyme A and B which should be kept on ice when not in use.

Enzyme A

Reconstitute Enzyme A (MAK442F) by adding 120 µL of purified water to the Enzyme A tube. Make sure Enzyme A is fully dissolved by pipetting up and down or with gentle vortexing. Reconstituted Enzyme A is stable for at least 1 week at -20 °C and can withstand up to 10x freeze-thaw cycles.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Sorbitol is soluble and readily extracted in water. Solid samples (food, fruits, etc.) can be homogenized in water followed by filtration using a 10 kDa spin column or centrifugation for 5 minutes at 14,000 × g. Beverage samples can be assayed directly.
2. Prior to assay, check the pH of the sample. If the pH is not between 7 and 8, adjust the sample pH to 7-8 with NaOH or HCl.
3. Remove protein from serum or plasma samples by using a 10 kDa spin column.
4. For unknown samples, test several dilutions to ensure that the readings are within the linear range of the Standard Curve.
5. All samples can be stored at -20 to -80 °C for at least one month.
6. Transfer 20 µL of each Sample to separate wells of a clear 96-well plate.
7. For visually colored Samples such as juices, prepare a Sample Blank by transferring an additional 20 µL of the Sample into a parallel well of the plate.

Standard Curve Preparation

1. Prepare a 1000 µM Sorbitol Standard by mixing 10 µL of the 50 mM Sorbitol Standard with 490 µL of purified water.
2. Prepare Sorbitol standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Sorbitol Standards

Well	1000 µM Sorbitol Standard	Purified Water	Sorbitol (µM)
1	250 µL	-	1000
2	150 µL	100 µL	600
3	75 µL	175 µL	300
4	-	250 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of the plate.

Working Reagent

Note: Fresh preparation of the Working Reagent is recommended. Use the Working Reagent within 60 minutes of preparation and keep protected from light.

Mix enough reagents for the number of assays to be performed. For each Standard and Sample well, prepare 85 µL of Working Reagent according to Table 2. If applicable, prepare 84 µL of Blank Working Reagent according to Table 2 for each Sample Blank well.

Table 2.
Preparation of Working Reagents

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	75 µL	75 µL
Enzyme A	1 µL	-
Enzyme B	1 µL	1 µL
NAD/MTT	8 µL	8 µL

Measurement

1. Add 80 µL of the Working Reagent to each Sample and Standard well.
2. If applicable, add 80 µL of the Blank Working Reagent to each Sample Blank well.
3. Tap plate to mix.
4. Incubate the plate for 30 minutes at room temperature.
5. Read optical density (OD) of all wells at 565 nm.

Results

1. Subtract the OD_{Blank} (Standard #4) reading from the OD readings for the remaining standards.
2. Plot the corrected Standard OD readings against the standard concentrations. Determine the slope of the Standard curve using linear regression.
3. Calculate the Sorbitol concentration of the sample:

$$\text{Sorbitol } (\mu M) = \frac{OD_{Sample} - OD_{Blank}}{\text{Slope } (\mu M^{-1})} \times DF$$

where

OD_{Sample} = OD value at 565 nm of Sample

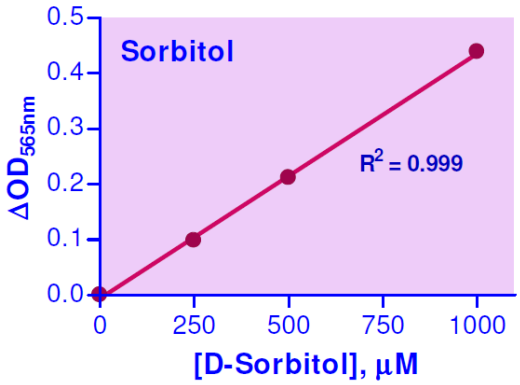
OD_{Blank} = OD value at 565 nm of Blank (Standard #4 or Sample Blank, if applicable)

DF = Sample Dilution Factor (DF= 1 for undiluted samples)

If the Sample sorbitol concentration exceeds 1000 µM, dilute the Sample in purified water and repeat the assay.

Conversions: 1 mM Sorbitol equals 18.2 mg/dL, 0.018% or 182 ppm.

Figure 1.
Typical Sorbitol Standard Curve



References

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2. Bailey, J.M., A microcolorimetric method for the determination of sorbitol, mannitol, and glycerol in biologic fluids. *J. Lab. Clin. Med*., 54(1):158-62 (1959).
3. Wolfson, S.K. Jr, and Williams-Ashman, H.G., Enzymatic determination of sorbitol in animal tissues. *Proc. Soc. Exp. Biol. Med.*, 99(3):761-5 (1958).

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