

Sorbitol Assay Kit (Colorimetric)

LS-K165-100 (100 Tests) • Store at -20°C



Introduction

SORBITOL (glucitol) is a sugar alcohol that is metabolized slowly in the human body. Sorbitol can be obtained from glucose by reducing 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 made solely 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. LSBio's sorbitol assay involves an end-point enzyme coupled MTT/NAD reaction that forms a colored product with an absorption maximum at 565 nm. The increase in absorbance at 565 nm is directly proportional to the sorbitol concentration.

Key Features

- Fast and sensitive. Linear detection range (20 µL sample): 5 to 1000 µM D-sorbitol.
- Convenient and high-throughput. Room temperature assay. No 37°C incubator required. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

- Sorbitol determination in biological (e.g. blood), food, beverage and agriculture samples.

Components

Component	K165-100
	100 Tests
Assay Buffer	10 mL
Enzyme A	120 µL
Standard (50 mM Sorbitol)	250 µL
NAD/MTT	1 mL
Enzyme B	120 µL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.

Storage

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

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Assay Procedure

Sample Preparation

Sorbitol is soluble and readily extracted in water. Solid samples (food, fruits etc.) can be homogenized in water followed by filtration or centrifugation (e.g. 5 min 14,000 rpm). Beverage samples can be assayed directly. 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. It is prudent to test several dilutions to determine an optimal dilution factor n .

Serum or plasma samples should be deproteinated using 10 kDa membrane filters (e.g. VWR cat # 82031-348).

All samples can be stored at -20 to -80°C for at least one month.

Procedure using 96-well plate

1. Standards. Prepare 500 μL 1000 μM . Premix by mixing 10 μL 50 mM Standard and 490 μL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table. Transfer 20 μL Standards into separate wells of a clear flat bottom 96-well plate.

No	Premix + H ₂ O	Sorbitol (μM)
1	250 μL + 0 μL	1000
2	150 μL + 100 μL	600
3	75 μL + 175 μL	300
4	0 μL + 250 μL	0

Samples. For visually colored samples such as juices, it is prudent to run a Sample Blank (see Step 2). Transfer 20 μL of the Sample in duplicate into two separate wells of the plate.

2. Equilibrate all reagents to room temperature. Briefly centrifuge tubes before use. Prepare enough Working Reagent (WR) for all standards and samples by mixing, for each well: 75 μL Assay Buffer, 1 μL Enzyme A, 1 μL Enzyme B and 8 μL NAD/MTT Solution. If including Sample Blanks, prepare a blank working reagent (BWR) without Enzyme A.

Note: Fresh reconstitution of the WR is recommended. Use the WR within 60 min. Keep the WR in dark if not used immediately.

3. Add 80 μL of the appropriate WR to each Standard and Sample well. Tap plate briefly to mix and incubate for 30 min at room temperature.
4. Read OD at 565nm (520-600nm) on a plate reader.

Calculation

Subtract blank value (water, #4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the Sorbitol concentration of Sample as follows:

$$[\text{Sorbitol}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

where $\text{OD}_{\text{SAMPLE}}$, OD_{BLANK} are optical density values of the Sample and H₂O Blank (or Sample Blank if a Sample Blank was needed), respectively. n is the sample dilution factor.

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

Note: If sample sorbitol concentration exceeds 1000 μM , dilute samples in water and repeat the assay. Multiply the results by the dilution factor.

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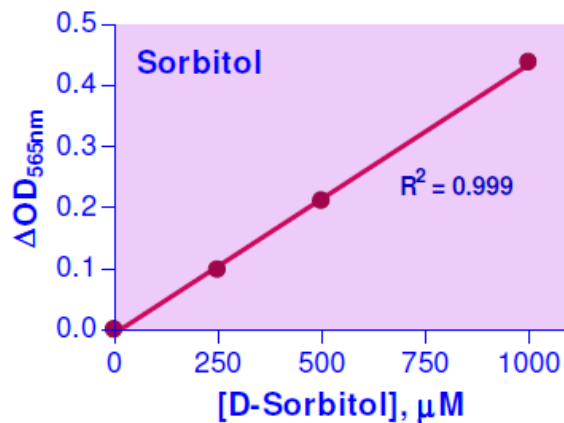
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Sample Data



Version: V.08.09.2018

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