EnzyChrom™ Sorbitol Assay Kit (ESBT-100)

Quantitative Colorimetric Sorbitol Determination

DESCRIPTION

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.

BioAssay Systems' 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.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**NAD/MTT:**	1 mL
Enzyme A:	120 µL	**Enzyme B:**	120 µL
Standard:	250 µL 50 mM Sorbitol		

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

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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_2O	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:

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

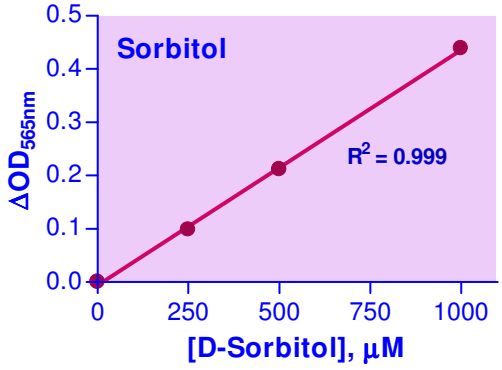
where OD_{SAMPLE}, OD_{BLANK} are optical density values of the Sample and H_2O Blank (or Sample Blank if a Sample Blank was needed), respectively. n is the sample dilution factor.

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

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.



LITERATURE

1. Gabbay, KH (1973). Role of sorbitol pathway in neuropathy. Adv Metab Disord. 2:Suppl 2: 417-32.

2. Bailey, JM. (1959). A microcolorimetric method for the determination of sorbitol, mannitol, and glycerol in biologic fluids. J Lab Clin Med. 54(1):158-62.

3. Wolfson, SK Jr, and Williams-Ashman, HG (1958). Enzymatic determination of sorbitol in animal tissues. Proc Soc Exp Biol Med. 99(3):761-5.