EnzyChrom™ D-Mannitol Assay Kit (EMNT-100)

Quantitative Colorimetric D-Mannitol Determination

DESCRIPTION

D-MANNITOL ($C_6H_{14}O_6$) is a sugar alcohol used in dietary supplement, sweetener, intestinal permeability test for leaky gut, etc. It also serves as a coating for hard candies, dried fruits, and chewing gums due to its low ability to attract and hold water molecules. In addition, it is an osmoprotectant for plants and is used clinically in osmotherapy to reduce intracranial pressure.

BioAssay Systems' D-mannitol assay kit is based on mannitol dehydrogenase catalyzed oxidation of D-mannitol, which generates D-fructose and NADH that reduces a formazan (MTT) dye. The intensity of product color, measured at 565 nm is directly proportional to D-mannitol concentration in the sample.

KEY FEATURES

Fast and sensitive. Use of 20 µL sample. Linear detection range 0.007 to 3 mM mannitol in 96-well plate assay.

Convenient. The procedure involves adding a single working reagent, and reading the absorbance after 30 minutes. Room temperature assay. No 37°C heater is needed.

High-throughput. "Add-mix-read" type assay. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

APPLICATIONS

Direct Assays: mannitol in food, beverage, agricultural products, and biological samples such as urine and serum.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 10 mL **Standard:** 0.5 mL 20 mM D-Mannitol

Enzyme: 120 µL

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. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. 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: clear and slightly colored samples can be assayed directly. It is prudent to test several dilutions to determine an optimal dilution factor *n*.

Solid samples can be homogenized in distilled water followed by filtration or centrifugation (e.g. 5-10 min 14,000 rpm).

Beverage samples can be assayed directly. Check the pH of the sample and adjust to 8-9 with NaOH or HCl if necessary. Samples containing carbon dioxide should be degassed by gentle stirring prior assay.

Biological fluid samples (e.g. urine & serum) can be assayed directly. Appropriate dilution in distilled water may be required.

Procedure using 96-well plate

1. *Standards*. Prepare 200 µL 3 mM Premix by mixing 30 µL of the Standard (20 mM) and 170 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + H_2O	D-Mannitol (mM)
1	100 µL + 0 µL	3.0
2	60 µL + 40 µL	1.8
3	30 µL + 70 µL	0.9
4	0 µL + 100 µL	0

2. Transfer 20 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 20 µL of each sample into two separate wells, one serving as a sample blank well (OD_{BLANK}) and one as a sample well (OD_{SAMPLE}).
3. Prepare sufficient Working Reagent (WR) for all sample and standard wells by mixing, for each well: 85 µL Assay Buffer plus 1 µL Enzyme.

   Add 80 µL WR to the *four Standards* and the *Sample Wells*. Add 80 µL Assay Buffer (*i.e. no Enzyme*) to the *Sample Blank Wells*. Tap plate to mix briefly and thoroughly. Incubate 30 minutes at room temperature.
3. Read optical density at 565 nm (520-600 nm).

CALCULATION

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

$$[\text{D-Mannitol}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (mM^{-1})} \times n \quad (mM)$$

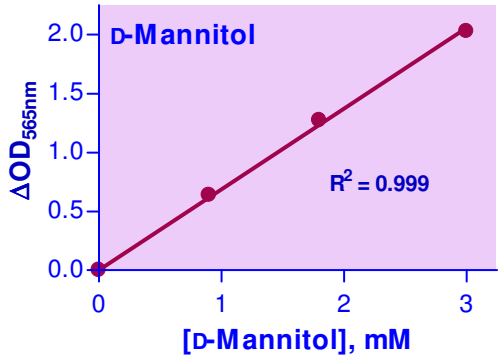
OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

Note: if the sample OD value is higher than OD for the 3 mM mannitol standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 mM D-mannitol equals 18.2 mg/dL, or 182 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

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



Standard Curve in 96-well plate assay in water

LITERATURE

1. Blood, Jane, et al (1991). Rapid enzymatic method for the measurement of mannitol in urine. Annals of Clinical Biochemistry: An international journal of biochemistry in medicine 28.4: 401-406.
2. Boaz, R. T., et al (2013). Intestinal Permeability in Normally Nourished and Malnourished Children with and without Diarrhea. Indian Pediatr 50: 152-153.
3. Sequeira, I. R., et al (2012). The effect of aspirin and smoking on urinary excretion profiles of lactulose and mannitol in young women: toward a dynamic, aspirin augmented, test of gut mucosal permeability. Neurogastroenterology & Motility 24, no. 9: e401-e411.