Technical Bulletin

Mannitol Assay Kit

Catalogue number MAK514

Product 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.

The 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.

The linear detection range of the kit is 0.007 to 3 millimolar (mM) mannitol. The kit is suitable for determination of mannitol in food, beverage, agricultural products, and biological samples such as urine and serum.

Components

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

- Assay Buffer 10 mL
  Catalogue Number MAK514A
- Enzyme 120 µL
  Catalogue Number MAK514B
- Standard (20 mM D-Mannitol) 0.5 mL
- Catalogue Number MAK514C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, 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
- If working with solid samples, Dounce tissue grinder set
- (Catalogue 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.

Procedure

Sample Preparation

All Samples and Standards should be run in duplicates. Clear and slightly colored liquid Samples can be assayed directly.

Solid Samples

1. Homogenize 200 mg Sample in 800 µL purified water.
2. Filter and centrifuge at 14,000 rpm for 5-10 min. Use the clear supernatant for the assay.

Beverage Samples

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

Biological Samples

Biological fluid Samples (For example. urine and serum) can be assayed directly. The appropriate dilution with purified water may be required.

Note: It is recommended to test several dilutions to determine an optimal dilution factor n.

Standard Curve Preparation

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

Table 1.

Dilution of Standard.

Well	3 mM Standard	Purified 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

Transfer 20 µL Standards into separate wells of a clear, flat-bottom 96-well plate.

Working Reagent

Prior to starting reaction, mix enough working reagent for the number of assays to be performed. Assay Buffer without enzyme will be used for the sample blank wells. Prepare working reagent according to Table 2.

Table 2.
Preparation of Working Reagent.

Reagent	Working Reagent
Assay Buffer	85µL
Enzyme	1 µL

Assay Reaction

1. Transfer 20 µL of each Standard and Sample into separate wells of a clear, flat-bottom 96-well plate.
2. Add 80 µL of the Working Reagent to the Standard and Sample wells.
3. Add 80 µL Assay Buffer to the Sample blank wells.
4. Tap plate to mix briefly and thoroughly.
5. Incubate for 30 minutes at room temperature.
6. Measure the optical density reading at 565 nm (520-600 nm).

Results

1. Calculate ΔOD by subtracting the blank reading Standard #4 (Blank) from the remaining Standard reading values.
2. Plot ΔOD against the standard concentrations.
3. Determine the slope and calculate the concentration of mannitol in the Sample using the following equation:

$$\text{D-Mannitol (mM)} = \frac{OD_{Sample} - OD_{Blank}}{\text{Slope}\left(mM^{-1}\right)} \times n$$

Where:

OD_{SAMPLE} = Optical Density reading of sample

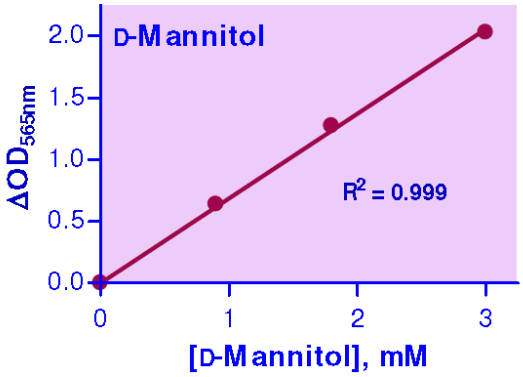
OD_{BLANK} = Optical Density reading of blank

n = Sample dilution factor (n = 1 for undiluted samples)

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.

Figure 1.
Typical D-Mannitol Standard Curve in 96-well plate assay in water.



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