



D-Mannitol Assay Kit (Colorimetric)

D-Mannitol Assay Kit (Colorimetric) is a detection kit for the quantification of D-Mannitol in Biological, food, beverage and agricultural products.

Catalog number: ARG82181

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Mannitol is a type of sugar alcohol used as a sweetener and medication. It is used as a low calorie sweetener as it is poorly absorbed by the intestines. As a medication, it is used to decrease pressure in the eyes, as in glaucoma, and to lower increased intracranial pressure. Medically, it is given by injection. Effects typically begin within 15 minutes and last up to 8 hours.

Common side effects from medical use include electrolyte problems and dehydration. Other serious side effects may include worsening heart failure and kidney problems. It is unclear if use is safe in pregnancy. Mannitol is in the osmotic diuretic family of medications and works by pulling fluid from the brain and eyes.

The discovery of mannitol is attributed to Joseph Louis Proust in 1806. It is on the World Health Organization's List of Essential Medicines. It was originally made from the flowering ash and called manna due to its supposed resemblance to the Biblical food. Mannitol is on the World Anti-Doping Agency's banned drug list due to concerns that it may mask other drugs. [Provide by Wikipedia: Mannitol]

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PRINCIPLE OF THE ASSAY

This D-Mannitol Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of D-maltose in food, beverage, agricultural product and biological samples. This assay 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 O.D. 565 nm is directly proportional to D-mannitol concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

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

| Component | Quantity | Storage information |
|-----------------------------|----------|---------------------|
| Assay Buffer | 10 mL | -20°C |
| Enzyme | 120 µL | -20°C |
| Standard (20 mM D-Mannitol) | 0.5 mL | -20°C |

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 565 nm
- Clear flat-bottom 96 well microplate
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Solid samples (E.g., food, fruits): homogenized in distilled water followed by filtration or centrifugation (E.g., 5 minutes at 14,000 rpm).

Beverage samples: assayed directly. Prior to assay, 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 (urine and serum): assayed directly. Appropriate dilution in distilled water may be required.

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REAGENT PREPARATION

- **Working Reagent:** for each well, mixing 1 μL of Enzyme and 85 μL of Assay Buffer. Fresh reconstitution is recommended.
- **Blank Working Reagent:** Use Assay Buffer as Blank Working Reagent.
- **Standards:** Mix 30 μL of 20 mM Standard with 170 μL of distilled water (final conc. 3 mM). Dilute Standards as follows;

| Standard tube | D-Mannitol (mM) | Distilled water (μL) | Standard Premix, 3 mM (μL) |
|---------------|-----------------|-----------------------------------|---|
| S1 | 3.0 | 0 | 100 |
| S2 | 1.8 | 40 | 60 |
| S3 | 0.9 | 70 | 30 |
| S4 | 0 | 100 | 0 |

ASSAY PROCEDURE

Equilibrate reagents to room temperature. Briefly centrifuge tubes before use.

| | Standard well | Sample well | Blank well |
|---|------------------|------------------|------------------|
| Each diluted Standard | 20 μL | | |
| Each Sample | | 20 μL | 20 μL |
| Working Reagent | 80 μL | 80 μL | |
| Blank Working Reagent | | | 80 μL |
| Tap plate to mix immediately. Incubate for 30 minutes at room temperature . | | | |
| Read the absorbance at O.D. 565 nm. (520-600 nm) | | | |

CALCULATION OF RESULTS

1. Subtract the blank value (S4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the D-Mannitol concentration of Sample,

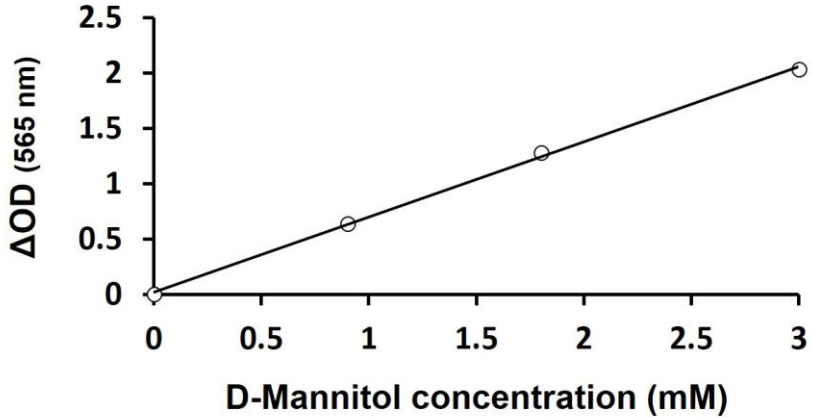
$$\text{D-Mannitol (mM)} = [(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{Slope (mM}^{-1}\text{)})] \times n$$

Note:

- $\text{OD}_{\text{Sample}}, \text{OD}_{\text{Blank}}$: the O.D. 565 nm values of the sample and blank.
 - n : the sample dilution factor.
2. Conversions: 1 mM D-mannitol equals 18.2 mg/dL or 182 ppm.
 3. If the sample OD value is higher than OD for the 3 mM mannitol standard, dilute sample in distilled water and repeat the assay. Multiply the results by the dilution factor.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the D-Mannitol Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

7 μM