Mannitol Assay Kit

Catalog No.: abx298897

Size: 96 tests

Detection range: 2 µg/ml – 200 µg/ml

Storage: Store all components at 4°C.

Application: For detection and quantification of Mannitol concentration in serum, urine, and other biological fluids.

Introduction

Mannitol is a simple sugar alcohol with a wide variety of uses in medicine, food production, and manufacturing. It is commonly used as a low-calorie sweetener due to its poor absorption in the gut compared to glucose, and its hydrophobic properties also make it useful as a preservative coating for chewing gums. In the medical field, Mannitol's ability to alter osmotic pressures when dissolved make it useful as a treatment for fluid build-up, such as in glaucoma, and as a first-line treatment for cerebral oedema following head trauma.

Abbexa's Mannitol Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Mannitol concentration. Any Mannitol present in the sample reacts with a colorimetric indicator to form a colored compound. The absorbance of this compound at 413 nm is proportional to the concentration of Mannitol in the sample. The concentration of Mannitol can be calculated by measuring the absorbance at 413 nm.

Kit components

1. 96-well microplate
2. Reaction Buffer: 2.5 ml
3. Dye Reagent: 10 ml
4. Stop Solution: 1 vial
5. Standard: 1 vial
6. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (413 nm)
2. Distilled water
3. Pipette and pipette tips
4. Centrifuge
5. Incubator
6. Microplate shaker

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

- **All fluids**: Prepare according to conventional methods. Samples can be tested directly.

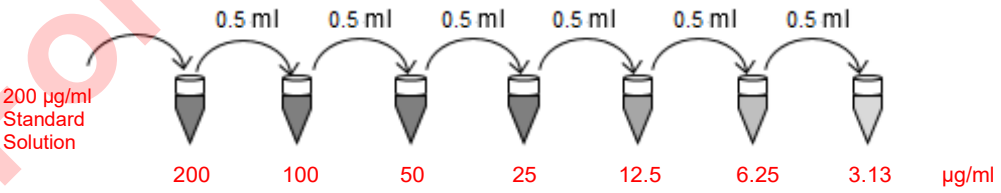
It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with distilled water, then carry out the assay procedure. The concentrations of Mannitol in each sample must fall within the kit's stated range (2 µg/ml – 200 µg/ml).

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

2. Reagents

- **Working Stop Solution**: Add 5 ml distilled water, and mix fully.
- **Mannitol Standard Dilutions**: Add 1 ml distilled water to the powdered Standard, and mix fully. In a fresh vial, add 0.9 ml distilled water, and then add 0.1 ml of the reconstituted Standard to create a 200 µg/ml stock standard solution. Label 7 tubes with 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, and 3.13 µg/ml. To all tubes except the first, add 0.5 ml distilled water. To the first tube, add 1 ml of the 200 µg/ml stock Standard. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



For the blank, or 0 µg/ml standard, use pure Distilled water.

B. Assay Procedure

1. Set the sample, standard, and blank wells on the microplate and record their positions. It is recommended to run each of these in duplicate, taking the mean OD of each pair of wells. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
2. Add 25 µl of sample to the sample wells.
3. Add 25 µl of each Standard Dilution to the standard wells.
4. Add 25 µl of distilled water to the blank wells.
5. Add 25 µl of Reaction Buffer to all wells.
6. Mix fully, and then allow to stand at room temperature for 10 minutes.
7. Add 50 µl of Working Stop Solution to all wells.
8. Mix fully, and then allow to stand at room temperature for 5 minutes.
9. Add 100 µl of Dye Reagent to all wells.
10. Mix fully with a microplate reader, seal, and incubate at 53°C for 15 minutes.
11. Immediately put on ice, and measure the OD of each well with a microplate reader at 413 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. This standard curve can be used to calculate the concentration of Mannitol in each sample.

Alternatively, based on this curve, the concentration of Mannitol in each sample well can be derived with the formula:

$$\text{Mannitol (µg/ml)} = C_{Standard} \times V_{Standard} \times \frac{(OD_{Sample} - OD_{Blank})}{V_{Sample} \times (OD_{Standard} - OD_{Blank})}$$

$$= 200 \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

where:

OD_{Sample}	:	Absorbance of the sample well at 413 nm
$OD_{Standard}$	:	Absorbance of the top standard well at 413 nm
OD_{Blank}	:	Absorbance of the blank well at 413 nm
$C_{Standard}$	:	Concentration of the top standard (200 µg/ml)
$V_{Standard}$	:	Volume of the top standard (0.025 ml)
V_{Sample}	:	Volume of the sample (0.025 ml)