Mannitol Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6760

Detection and Quantification of Mannitol Content in Serum, Urine, Other biological fluids, Food, Beverage Samples.

For research use only . Not for diagnostic or therapeutic procedures .

1. Introduction

Mannitol 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.

Mannitol Colorimetric Microplate Assay Kit is designed to measure mannitol in various samples in 96 -well microplate. The color intensity at 413 nm is directly proportional to mannitol concentration in the sample.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Reaction Buffer	2.5 mL x 1	4 °C
Stop Solution	Powder x 1	4 °C
Dye Reagent	10 mL x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Microplate reader to read absorbance at 413 nm
2. Distilled water
3. Pipettor , multi-channel pipettor
4. Pipette tips
5. Distilled water
6. Hot air circulation oven

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL distilled water to generate 2 mg/ml of standard stock/top solution, store at 4°C for 1 -2 months after reconstitution. Then dilute to 200 μg/ml standard top solution by adding 0.1 mL stock solution into 0.9 mL distilled wa ter. Perfo rm 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 200/100/50/25/12.5/6.25/3.12 μg/ml.

Stop Solution: Add 5 mL distilled water to dissolve before use. Store at 4°C for 3 months after reconstitution.

5. Sample Preparation

1. For serum, urine and o ther biological fluids samples Samples can be assayed directly.

6. Assay Procedure

Add following reagents in the microplate :

Reagent*	Sample**	Standard	Blank
Sample	25 μl	--	--
Standard	--	25 μl	--
Distilled water	--	--	25 μl
Reaction Buffer	25 μl	25 μl	25 μl
Mix, incubate at room temperature for 10 minutes.			
Stop Solution	50 μl	50 μl	50 μl
Mix, incubate at room temperature for 5 minutes.			
Dye Reagent	100 μl	100 μl	100 μl
Mix, cover the plate adhesive strip, put the plate into the oven, incubate at 53 °C for 15 minutes, then put it on ice immediately, record absorbance measured at 413 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (\mu g/ml)$$

Calculate the initial concentration according to sample preparation procedure .

2. According to one point of the standard OD and concentration

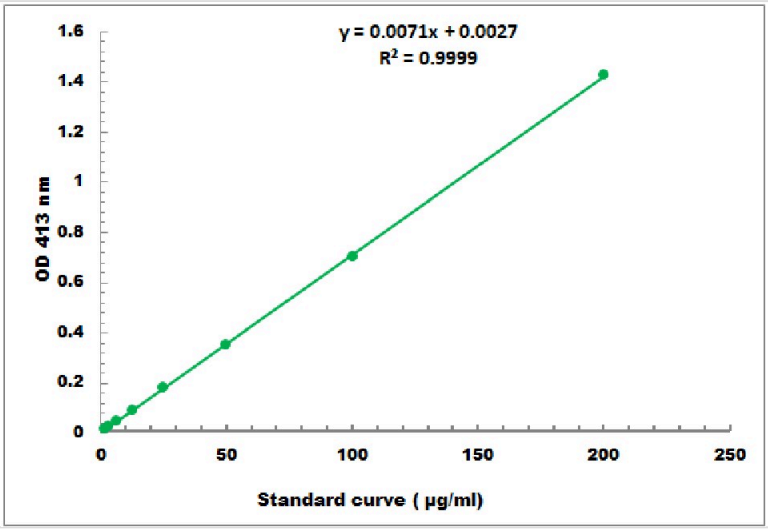
2.1 According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \ (\mu g/ml)$$

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Standard}$	the standard concentration, μg/ml
$V_{Standard}$	the volume of standard in assay procedure, μl
V_{Sample}	the volume of sample in assay procedure, μl

8. Typical Data

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range : 2 μg/ml - 200 μg/ml