Mannitol

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1165

(Version 1.4A)

Detection and Quantification of MannitolContentin Serum, Urine,

Other biological fluids, Food, BeverageSamples.

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

I. INTRODUCTION

Mannitol is a sugar alcohol used in dietary supplement,sweetener, intestinal permeability test for leaky gut, etc. It also serves asa coating for hard candies, dried fruits, and chewing gums due to its lowability to attract and hold water molecules. In addition, it is anosmoprotectant for plants and is used clinically in osmotherapy to reduceintracranial pressure.

MannitolColorimetric Microplate Assay Kitis designed to measure mannitol in various samples in 96-well microplate. The color intensity at 413 nm is directly proportional to mannitolconcentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	2.5 mlx 1	4 °C
Stop Solution	Powderx 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	

III. 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

IV. REAGENT PREPARATION

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

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

V. SAMPLE PREPARATION

1.Forserum, urine and other biological fluidssamples

Samples can be assayed directly.

VI. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent*	Sample**	Standard	Blank
Sample	25µl	--	--
Standard	--	25µl	--
Distilled water	--	--	25µl
Reaction Buffer	25µl	25µl	25µl
Mix, incubate atroom temperature for 10minutes.			
Stop Solution	50 µl	50 µl	50 µl
Mix, incubate atroom temperature for 5minutes.			
Dye Reagent	100 µl	100 µl	100 µl
Mix, cover the plate adhesive strip, put the plate into the oven, incubate at53 °Cfor 15minutes, 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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe 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

2.1According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

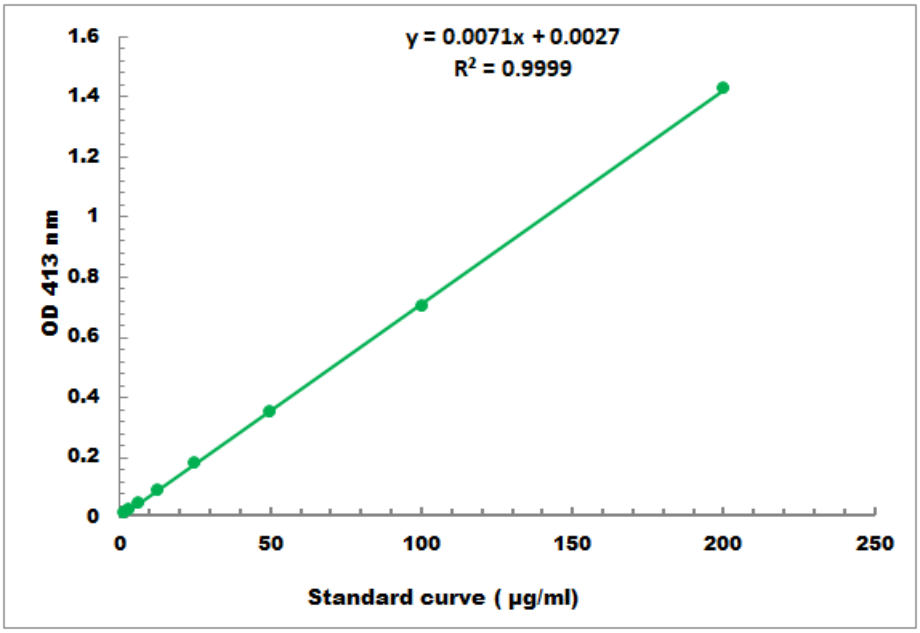
$C_{Standard}$: the standard concentration, μg/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

VIII. TYPICAL DATA

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



Detection Range: 2µg/ml - 200µg/ml