3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

D-Mannitol Colorimetric Assay Kit

Catalog Number **MAK096**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

D-Mannitol is a common sugar alcohol (polyol) produced by many organisms including bacteria, fungi, and plants. D-Mannitol serves as a carbon storage compound, an osmoregulating agent, and antioxidant by protecting against reactive oxidative species (ROS). Mannitol is commonly used in food manufacturing as a reduced-calorie sweetener.

The D-Mannitol Colorimetric Assay kit provides a simple and direct procedure for measuring D-mannitol in a variety of samples, including fruit and juices. D-Mannitol concentration is determined by a coupled enzyme assay, which results in a colorimetric (450 nm) product proportional to the D-mannitol present.

The sugar alcohol L-arabitol also acts as a substrate for this assay; therefore, this kit can be used to measure L-arabitol.

Components

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

Mannitol Assay Buffer Catalog Number MAK096A	27 mL
Mannitol Enzyme Mix Catalog Number MAK096B	1 vl
Mannitol Substrate Mix Catalog Number MAK096C	1 vl
Mannitol Standard Catalog Number MAK096D	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Mannitol Assay Buffer – Allow buffer to come to room temperature before use.

Mannitol Enzyme Mix – Reconstitute with 190 µL of Mannitol Assay Buffer. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at 2–8 °C. Use within 2 months of reconstitution and keep cold while in use.

Mannitol Substrate Mix – Reconstitute with 220 µL of water. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at 2–8 °C. Use within 2 months of reconstitution.

Mannitol Standard – Reconstitute with 100 µL of water to generate a 100 mM standard solution. Mix well by pipetting, then aliquot and store, protected from light, at 2–8 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability

The kit is shipped on wet ice and storage at 2–8 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate. Use ultrapure water for the preparation of standards.

Mannitol Standards for Colorimetric Detection
Dilute 10 μL of the 100 mM Mannitol Standard solution with 990 μL of water to prepare a 1 mM (1 nmol/μL) standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Mannitol Assay Buffer to each well to bring the volume to 50 μL.

Sample Preparation
Tissue (100 mg), cells (4×10^6), or fruit can be homogenized in 200 μL of ice-cold Assay Buffer. Centrifuge the samples at 13,000 × *g* for 5 minutes to remove insoluble material. Transfer the supernatant to the appropriate well plate.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Bring samples to a final volume of 50 μL in each well with Mannitol Assay Buffer. Run samples in duplicate wells.

NADH present in the sample can generate background. To control for NADH background, include a blank sample for each sample by omitting the Mannitol Enzyme Mix in the Reaction Mix.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 μL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	**Samples and Standards**	**Blank Sample**
Mannitol Assay Buffer	46 μL	48 μL
Mannitol Enzyme Mix	2 μL	–
Mannitol Substrate Mix	2 μL	2 μL

2. Add 50 μL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 20 minutes at 37 °C. Cover the plate and protect from light during the incubation.

3. Measure the absorbance at 450 nm (A_{450}).

Results

Calculations

The background for the assay is the value obtained for the 0 (blank) mannitol standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate mannitol standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of D-mannitol present in the samples may be determined from the standard curve.

Concentration of D-Mannitol

$$S_a/S_v = C$$

S_a = Amount of mannitol in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added to reaction well

C = Concentration of Mannitol in sample

D-Mannitol molecular weight: 182.2 g/mole

Sample Calculation

Amount of D-Mannitol (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of D-Mannitol in sample

$$5.84 \text{ nmole}/50\ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

$$0.1168 \text{ nmole}/\mu\text{L} \times 182.2 \text{ ng/nmole} = 21.3 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

KHS,MF,LS,MAM 08/18-1