Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

D-Mannitol Colorimetric Assay Kit

rev.12/17

(Catalog # K644-100; 100 assays; Store at 4 °C)

I. Introduction:

D-Mannitol is one of the most abundant sugar alcohols in nature. It can be produced in the biosphere by a range of organisms such as bacteria, yeast, fungi and almost all plants. Mannitol functions as a carbohydrate storage depot, as a scavenger for reactive oxygen species and as an osmoprotectant. Recent studies have shown that increased mannitol content in plants protects them against fungal infections and improves resistance to drought and high salinity. The mannitol pathway reversibly converts fructose to mannitol and serves as the main carbohydrate cycle for fungi survival. Measurement of mannitol level is important for evaluating mannitol metabolic pathways and commercially, in developing fungi and drought resistant plants. In BioVision's D-Mannitol Assay Kit, D-mannitol is converted to D-fructose by mannitol dehydrogenase in the presence of NAD to form NADH, which reduces a colorless probe to a chromogen with strong absorbance at 450 nm. L-Arabitol, another sugar alcohol, also acts as substrate for mannitol dehydrogenase & undergoes similar reaction; therefore, this kit can also be used to measure L-arabitol. The D-mannitol assay kit is fast (~20 minutes), sensitive & easy to use. This assay kit can detect mannitol level less than 1 nmol/reaction (< 10 µM) & can be used for a variety of sample types.

D-Mannitol + NAD $\xrightarrow{\text{Mannitol Dehydrogenase}}$ D-Fructose + NADH $\xrightarrow{\text{Probe}}$ Color detection (λ = 450 nm)

L-Arabitol + NAD $\xrightarrow{\text{Mannitol Dehydrogenase}}$ L-Xylulose + NADH $\xrightarrow{\text{Probe}}$ Color detection (λ = 450 nm)

II. Application:

- Measurement of D-Mannitol/L-Arabitol in various plant tissues/cells and food products.
- Analysis of mannitol metabolic pathways and cell signaling in various organisms.

III. Sample Type:

Plant tissues, fungi, bacteria, food, juices, other beverages, etc.

IV. Kit Contents:

Components	K644-100	Cap Code	Part Number
Mannitol Assay Buffer	25 ml	WM	K644-100-1
Mannitol Enzyme Mix	30 µl	Green	K644-100-2
Mannitol Substrate Mix (Lyophilized)	1 vial	Red	K644-100-3
Mannitol Standard (Lyophilized)	1 vial	Yellow	K644-100-4

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Store kit at 4 °C, protected from light. Warm all Buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

- **Mannitol Enzyme Mix**: Dilute further by adding 190 µl Assay Buffer. Pipette up and down to mix completely. Aliquot & store at 4 °C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.
- **Mannitol Substrate Mix**: Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve completely. Stable for 2 months at 4 °C.
- **Mannitol Standard:** Reconstitute with 100 µl dH_2O to generate 100 mM Mannitol Standard solution. Keep on ice while in use. Store at 4 °C. Use within two months.

VIII. D-Mannitol Assay Protocol:

1. **Standard Curve Preparation:** Dilute Mannitol Standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM Mannitol Standard to 990 µl dH_2O & mix well. Add 0, 2, 4, 6, 8, and 10 µl of the 1 mM Mannitol Standard into a series of wells in a 96 well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well of Mannitol Standard. Adjust volume to 50 µl/well with Assay Buffer.
2. **Sample Preparation:** Homogenize plant cells/tissues (100 mg) or fruits on ice with 200 µl ice cold Mannitol Assay Buffer. Centrifuge at 12,000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (400 µg) per well and adjust the final volume to 50 µl with Mannitol Assay Buffer. Prepare a parallel sample well as the background control to subtract interference from NADH in the samples. **Notes:** For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
3. **Reaction Mix:** Mix enough reagents for the number of assays (samples and Standards) to be performed. For each well, prepare 50 µl reaction mix containing:

	Reaction Mix	Background Control Mix
Mannitol Assay Buffer	46 µl	48 µl
Mannitol Enzyme Mix	2 µl	----
Mannitol Substrate Mix	2 µl	2 µl

Add 50 µl of the reaction mix to each well containing the Standard and test samples and 50 µl of background control mix to each well containing the background control sample. Mix well.

4. **Measurement:** Incubate for 20 min at 37°C and measure OD_{450nm}.
5. **Calculation:** Subtract the 0 Standard reading from all Standard readings. Plot the Mannitol Standard Curve. Correct sample background by subtracting the value derived from the background control from sample readings. Apply the corrected sample readings to Standard Curve to get B nmol of Mannitol amount in the sample wells.

   The Mannitol concentration in the sample:

C = B/V x Dilution Factor = nmol/ml = µM

Where: **B** is the amount of Mannitol from the Standard curve (nmol)

V is the sample volume added into reaction well (ml).

Mannitol molecular weight: 182.17 g/mol

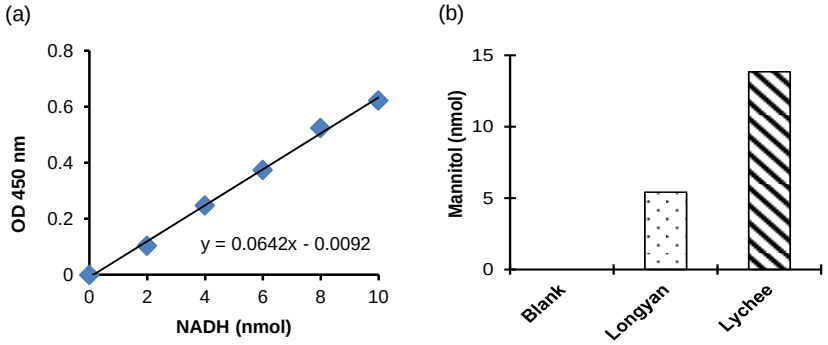

Figure. Mannitol Standard Curve (a). Measurement of Mannitol in the extracts of Longyan & Lychee (b). Assays were performed following kit protocol.

IX. RELATED PRODUCTS:

Glucose Assay kit
Glucose Dehydrogenase Activity Assay Kit
Glucose-1-Phosphate Colorimetric Assay Kit
Glucose Uptake Colorimetric Assay Kit
Glycogen Assay Kit
Maltose and Glucose Assay Kit
NADP/NADPH Quantification Kit
Phosphoglucose Isomerase Colorimetric Assay Kit
Glucose and Sucrose Assay Kit
Glucose-6-Phosphate Dehydrogenase Assay Kit
PicoProbe™ Glucose-6-Phosphate Assay Kit
Glucose Uptake Fluorometric Assay Kit
Hexokinase Colorimetric Assay Kit
NAD/NADH Quantification Kit
Phosphoglucomutase Colorimetric Assay Kit
Starch Assay Kit

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