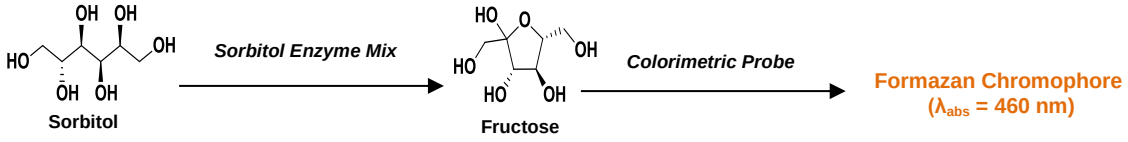
D-Sorbitol Assay Kit II (Colorimetric)

11/21

(Catalog # K2119-100; 100 assays; Store at -20 °C)

I. Introduction:

Sorbitol (D-glucitol) is a 6-carbon sugar alcohol naturally produced in plants, obtained by the reduction of the aldehyde moiety of glucose. It is one of the main photosynthetic end products and serves as a storage and transport sugar in most plant families, and plays an important role in osmotic adjustment in cell cytoplasm under various abiotic stresses such as salinity, chilling, and drought. It is found in abundance in fruits such as apples, berries and stone fruits (e.g. peaches, apricots and plums). Sorbitol is commonly used as an artificial sweetener, as a laxative and in cosmetics as a humectant and thickening agent. It can be produced in humans in small amounts by the reduction of glucose by aldose reductase. Due to its poor ability to diffuse across the cell membrane, sorbitol can become trapped in cells and is believed to be one of the causes of neuropathic damage (due to osmotic effects) in diabetes. Interestingly, sorbitol can be used as a screen for the O154:H7 strain of *E. coli*, since this strain is one of the few strains which cannot metabolize sorbitol. **BioVision's D-Sorbitol Assay Kit II** provides a quick and easy way to determine accurately the amount of sorbitol in a variety of samples such as foods, fruits, beverages, pharmaceuticals, cosmetics and other biological samples. In this Assay, sorbitol is oxidized to fructose, with the proportional reduction of a colorimetric probe that has an absorption maximum at 460 nm. The assay is simple, rapid and can detect a minimum of 0.1 nmoles/well of sorbitol (~5 µM in a 20 µl sample volume).



II. Application:

- Measurement of sorbitol in foodstuffs, beverages, plant extracts, etc.

III. Sample Types:

- Food products (fruits, vegetables), beverages, cosmetics and pharmaceuticals
- Plant extracts (natural or herbal products)

IV. Kit Contents:

Components	K2119-100	Cap Code	Part Number
Sorbitol Assay Buffer	25 ml	WM	K2119-100-1
Sorbitol Probe	1 vial	Red	K2119-100-2
Sorbitol Enzyme Mix	1 vial	Green	K2119-100-3
Sorbitol Standard (100 mM)	100 µl	Yellow	K2119-100-4

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

- **Sorbitol Assay Buffer:** Allow to equilibrate to room temperature (RT) prior to use. Buffer may be stored at +4 °C or -20 °C.
- **Sorbitol Probe (lyophilized) and Sorbitol Enzyme Mix (lyophilized):** Reconstitute each vial with 220 µl Sorbitol Assay Buffer. Pipette up and down to dissolve completely. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles. Use within two months following reconstitution.
- **Sorbitol Standard (100 mM):** Allow to equilibrate to RT. Vortex briefly and spin down. Divide into aliquots, if desired and store at -20 °C. Stable for at least 3 freeze/thaw cycles.

VII. Assay Protocol:

1. **Sample Preparation:** A variety of fruit, vegetable and plant samples, beverages as well as herbal/natural products can be analyzed with this assay. For solid samples such as fruit flesh and plant material, homogenize the sample in Sorbitol Assay Buffer (10-50 mg of sample per 100 µl buffer) using a Dounce homogenizer (BioVision Cat# 1998) or probe sonicator. Centrifuge the homogenate at 10000 x *g* and RT for 10 min and transfer the supernatant to a new microfuge tube. Liquid samples such as juices should be diluted with Sorbitol Assay Buffer at 1:10 ratio and centrifuged at 10000 x *g* and RT for 10 min to pellet any insoluble material.

   Notes:

   - We recommend running a parallel Sample Background Control well, in case the sample contains interfering substances that may generate background.
   - Samples with unknown quantities of sorbitol should be run at varying dilutions to ensure that the readings fall within the linear portion of the Sorbitol Standard Curve. Samples that are outside of the Standard Curve range should be diluted with Sorbitol Assay Buffer and re-tested.
   - This assay is not recommended for plasma, serum or urine samples (normal physiological plasma sorbitol levels are below the assay detection limit).

2. **Sorbitol Standard Curve:** Dilute the Sorbitol Standard to 1 mM by mixing 10 µl of Sorbitol Standard and 990 µl of dH_2O. Add 0, 2, 4, 6, 8 and 10 µl into a series of wells in a 96-well clear plate. Adjust the volume to 50 µl/well with Sorbitol Assay Buffer to generate the Sorbitol Standard Curve with 0, 2, 4, 6, 8 and 10 nmoles/well of the Sorbitol Standard.
3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Control Mix
Sorbitol Assay Buffer	46 µl	48 µl
Sorbitol Probe	2 µl	2 µl
Sorbitol Enzyme Mix	2 µl	—

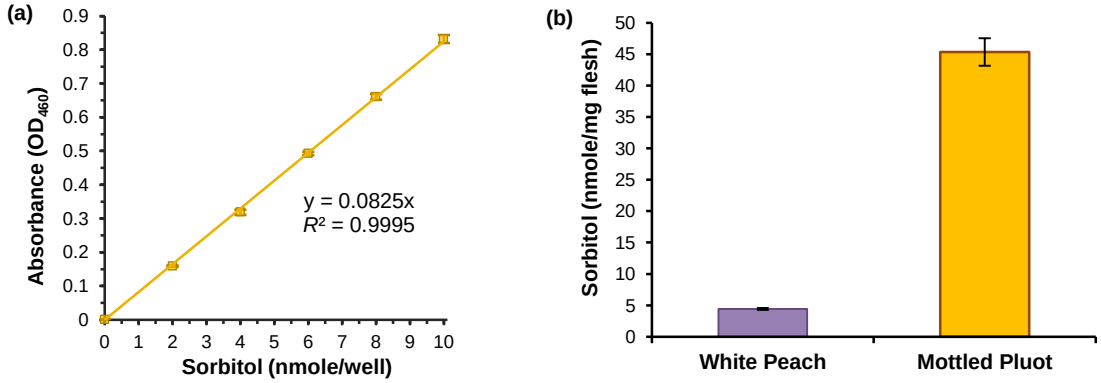
Add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control and test samples and 50 µl of Background Control Mix to each Sample Background Control well.

4. **Measurement:** Incubate the plate for 30 min at 37 °C, protected from light. Measure the absorbance (OD) at 460 nm in a microplate reader in endpoint mode.
5. **Calculation:** Subtract 0 Standard reading from all Standard readings and plot the background-subtracted values and calculate the slope of the Sorbitol Standard Curve. For test samples, calculate the corrected sample absorbance (*C*) by subtracting the Sample Background Control value (if applicable) from the Sample absorbance. Apply the *C* values to the Sorbitol Standard Curve to get *B* nmoles of Sorbitol in the well.

Sample Sorbitol Concentration $C = \frac{B}{V} \times D$ **= nmole/µl ≡ mM**

Where: ***B*** is the amount of sorbitol (in nmoles) calculated from the Standard Curve
V is the sample amount (in µl or mg) added into the reaction well
D is the sample dilution factor (if applicable, *D*= 1 for undiluted samples)

Molecular Weight of D-Sorbitol = 182.17 g/mol



Figures: (a) Sorbitol Standard Curve. **(b)** Quantification of sorbitol content in the flesh of white peach and mottled pluot. Fruit samples were homogenized in Sorbitol Assay Buffer and sorbitol levels are reported as nmoles per mg of fruit. For each sample, 4 µl of diluted homogenate (peach homogenate was diluted 5-fold and pluot was diluted 40-fold in Sorbitol Assay Buffer) was added per well. Mean sorbitol concentrations were 4.43 ± 0.16 and 45.37 ± 2.19 nmole/mg, respectively. Data are mean ± SD of 3 replicates, assayed according to the kit protocol.

VIII. Related Products:

Glucose and Sucrose Assay Kit (K616)
Glucose Uptake Colorimetric Assay Kit (K676)
Glucose Uptake Fluorometric Assay Kit (K666)
Sorbitol Dehydrogenase Activity Assay Kit (K935)
PicoProbe™ Glucose-6-Phosphate Assay Kit (K687)
Phosphoglucomutase Assay Kit (K774)
D-Sorbitol Colorimetric Assay Kit (K631)
Glucose-6-Phosphate Dehydrogenase Assay Kit (K757)
Fructose Assay kit (K619)
Hexokinase Assay Kit (K789)
Glucose Dehydrogenase Activity Assay Kit (K679)
Free Glycerol Colorimetric Assay Kit (K634)

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