BioVision

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For research use only

D-Sorbitol Colorimetric Assay Kit

(Catalog #K631-100; 100 assays; Store kit at -20 °C)

I. Introduction:

Sorbitol is one of the 6 carbon sugar alcohols. It is commonly used as an artificial sweetener, as a laxative and in cosmetics as a humectant and thickening agent. Sorbitol is produced naturally in a variety of fruits. 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 be trapped in cells and is believed to be one of the causes of 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 Sorbitol Colorimetric Assay Kit** is designed to measure sorbitol in a variety of samples such as **foods, fruits, fruit juices, pharmaceuticals, cosmetics, paper.** *This assay is not recommended for plasma, serum or urine samples.* In the assay, sorbitol is oxidized to fructose with the proportional development of intense color with an absorbance maximum at 560 nm. The assay is useful over the range of 0.1-10 nmol of Sorbitol per sample.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Sorbitol Assay Buffer	25 ml	WM	K631-100-1
Sorbitol Probe	200 µl	Red	K631-100-2
Sorbitol Enzyme Mix	lyophilized	Green	K631-100-3
Sorbitol Developer	lyophilized	Blue	K631-100-4
Sorbitol Standard (100 mM)	100 µl	Yellow	K631-100-5

III. Storage, Handling and General Consideration:

Store the kit at -20 °C and protect from light. Please read the entire protocol before performing the assay. **Avoid repeated freeze/thaw cycles as they will inactivate the components.**

IV. Reagent preparation:

Sorbitol Enzyme Mix: Add 220 µl dH_2O and dissolve well. The enzyme mix is stable at 4 °C for at least two weeks. If it is anticipated that reconstituted enzyme will be needed for a longer period, it should be aliquoted into small portions and stored frozen at -20 °C.

Sorbitol Developer: Add 1 ml dH_2O and dissolve well. Keep on ice while using. Store at 4 °C for short term storage (<2 weeks); store at -20 °C for longer term storage. Avoid multiple freeze/thaw cycles. If kit will be used multiple times over an extended period of time, divide into aliquots and store at -20 °C.

V. Assay Protocol:

1. **Standard Curve Preparation:**

Dilute the Sorbitol Standard to 1.0 mM by adding 10 µl of the Standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 96 well plate. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Sorbitol Standard.

2. **Sample Preparation and Consideration:**

Samples such as **food products and pharmaceuticals** should be dissolved in dH_2O, then centrifuge to spin down any insoluble materials. **Liquids such as juice** should be diluted with dH_2O 1:9 and centrifuged. Samples with unknown quantities of sorbitol should be run at varying dilutions to ensure that the reading fall within the linear portion of the standard curve. If samples containing high levels of interfering substances are to be analyzed, a background control can be performed, and run in parallel, in the absence of the enzyme mix. **Note:** This assay is not recommended for plasma, serum or urine samples.

3. **Reaction Mix Preparation:** Prepare 50 µl of Reaction Mix for each well to be measured including all standard, sample and background wells. For each well use:

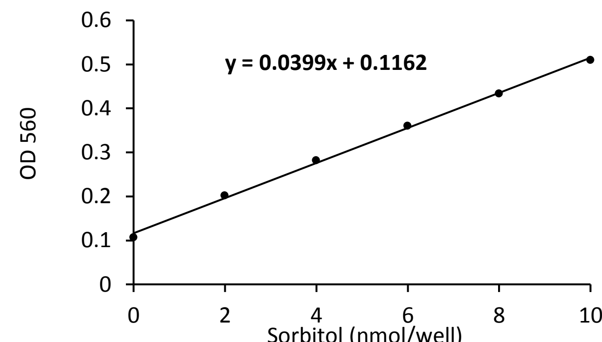
	Sample	Background
Sorbitol Assay Buffer	36 µl	38 µl
Sorbitol Enzyme Mix	2 µl	-----
Sorbitol Developer	10 µl	10 µl
Sorbitol Probe	2 µl	2 µl

Add 50 µl of the Reaction Mix into each well.

4. **Incubation:** 30 min at 37 °C.
5. **Read:** Measure OD at 560 nm in a microplate reader **with gentle shaking for 2 sec between measurements.**
6. **Calculation:** Correct background by subtracting the value derived from the 0 Sorbitol Standard from all readings. Note: The background reading can be significant and must be subtracted. Plot the Sorbitol Standard Curve. If samples have parallel background wells, subtract the value of each sample background well from each sample well. Read sample amount from the Standard Curve. Sorbitol concentration in samples:

$$C = S_a/S_v * D \quad \text{nmol/µl or mM}$$

Where: S_a is the sample amount (in nmol) from standard curve.
S_v is the sample volume (µl) added into the reaction wells.
D is the sample dilution factor if any.
D-Sorbitol MW: 182.17 g/mol.



RELATED PRODUCTS:

NAD(P)/NAD(P)H Quantification Kit	ADP/ATP Ratio Assay Kit
Ascorbic Acid Quantification Kit	Glutathione Detection Kits
Glucose Assay Kit	Fructose Assay Kit
Fatty Acid Assay Kit	Ethanol Assay Kit
Uric Acid Assay Kit	Alanine Assay Kit
Pyruvate Assay Kit	Lactate Assay Kit/ II
Triglyceride Assay Kit	Phosphate Assay Kit
Choline/Acetylcholine Quantification Kit	Hemin Assay Kit
Antioxidant Capacity (TAC) Assay Kit	Glycogen Assay Kit
L-Amino Acid Assay Kit	Nitric Oxide Assay Kits

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		