Product Information

D-Sorbitol Colorimetric Assay Kit

I. Kit Contents:

Components	K2135-100 100 assays	Cap Color	Part Number
Sorbitol Assay Buffer	25 ml	WM	K2135-C-1
Sorbitol Probe	200 µl	Red	K2135-C-2
Sorbitol Enzyme Mix	lyophilized	Green	K2135-C-3
Sorbitol Developer	lyophilized	Blue	K2135-C-4
Sorbitol Standard (100 mM)	100 µl	Yellow	B2029

II. Introduction:

Sorbitol is a sugar alcohol and often used as an artificial sweetener and a laxative. Sorbitol can also be used as a humectant and thickening agent in cosmetics. Sorbitol is naturally produced in a variety of fruits and can be produced in small amounts in humans via the reduction of glucose by aldose reductase. Sorbitol can be used to screen the O154:H7 strain of E. coli, since it cannot metabolize sorbitol. Because of its poor ability to diffuse across the cell membrane, sorbitol is one of the causes of damage in diabetes.

The D-Sorbitol Colorimetric Assay Kit provides a simple, fast and convenient way for detection of sorbitol in various samples such as fruit juices, fruits, foods, pharmaceuticals, cosmetics, paper and some other biological samples based on colorimetric method. In the assay, sorbitol is oxidized to fructose with the proportional intense color (λ = 560 nm) development. The kit can detect 0.1 - 10 nmol sorbitol per sample.

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, aliquot portions and freeze.

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 insolubles. 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. This assay is not recommended for plasma, serum or urine samples.

3. Reaction Mix: Prepare 50 µl of Reaction Mix for each well to be measured (All standard, sample and background wells). For each well use:

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

6. Calculation: Correct background by subtracting the value derived from the 0 Sorbitol Standard from all readings (The background reading can be significant and must be subtracted). Plot the Standard Curve. If samples have parallel background wells, subtract the value of each background well from each sample well. Read sample amount from the standard curve. Sorbitol concentration in samples:

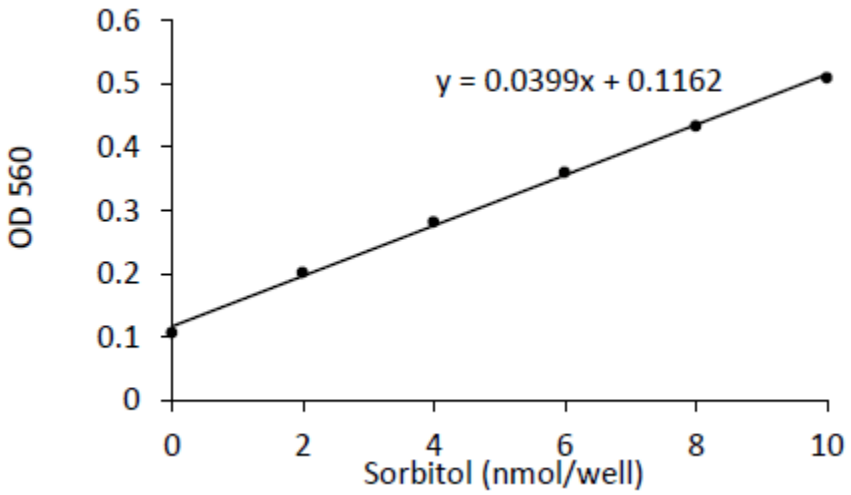
C = Sa/Sv * D nmol/µl or mM

Where: Sa is the sample amount (in nmol) from standard curve.

Sv 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.



General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different 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 d atasheet) • 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 data sheet & 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.		

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com