ab118968

D-Sorbitol Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of D-Sorbitol levels in various samples.

This product is for research use only and is not intended for diagnostic use.

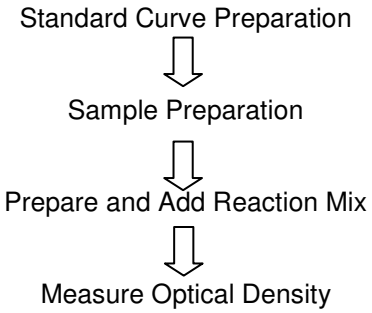
Table of Contents

1. Overview

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.

Abcam's D-Sorbitol Colorimetric Assay Kit is designed to measure sorbitol in a variety of samples such as foods, fruits, fruit juices, pharmaceuticals, cosmetics, paper and some other biological 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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Sorbitol Assay Buffer	25 mL
Sorbitol Probe	0.2 mL
Sorbitol Enzyme Mix (Lyophilized)	1 vial
Sorbitol Developer (Lyophilized)	1 vial
Sorbitol Standard (100mM)	100 µL

Store the kit at -20℃, protect from light. Read the entire protocol before performing the assay. **Avoid repeated freeze/thaw cycles as they will inactivate the components.**

SORBITOL ENZYME MIX: Add 220 µl dH_2O and dissolve well. The enzyme mix is stable at +4℃ 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℃.

SORBITOL DEVELOPER: Add 1 ml dH_2O and dissolve well. Keep on ice while using. Store at +4℃ for short term storage (<2 weeks); store at -20℃ 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.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96-well plate
- Orbital shaker

4. 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:

Samples such as food products and pharmaceuticals should be dissolved in dH_2O, and then centrifuged 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.

Notes:

a) If samples containing high levels of interfering substances are to be analyzed, a background control (in the absence of the enzyme mix) can be performed, and run in parallel.

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

3. Sorbitol Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

	Sample	**Background Control**
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 to each well containing the Sorbitol Standard and test samples. Add 50 µl of the Background Control Mix to sample control well. Mix well. Incubate the reaction for 60 min at 37℃, protect from light.

4. Measure OD_{560nm} in a microplate reader

5. Data Analysis

Correct background by subtracting the value derived from the zero 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:

Concentration = Sa / Sv x D (nmol/µl or mM)

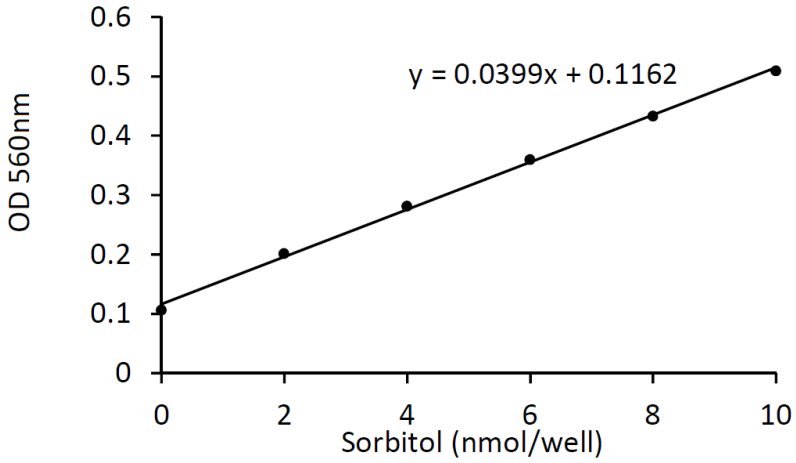
Where:

Sa is the sample amount of unknown Sorbitol (in nmol) from standard curve.

Sv is sample volume (µl) added into the wells.

D is the sample dilution factor (if any)

D-Sorbitol Molecular Weight is 182.17 g/mol.



Sorbitol Standard Curve: Performed according to assay protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

UK, EU and ROW
Email: technical@abcam.com
Tel: +44 (0)1223 696000
www.abcam.com

US, Canada and Latin America
Email: us.technical@abcam.com
Tel: 888-77-ABCAM (22226)
www.abcam.com

China and Asia Pacific
Email: hk.technical@abcam.com
Tel: 108008523689 (中國聯通)
www.abcam.cn

Japan
Email: technical@abcam.co.jp
Tel: +81-(0)3-6231-0940
www.abcam.co.jp