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Product Information

D-Sorbitol Colorimetric Assay Kit

Catalog Number **MAK010**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

D-Sorbitol is a sugar alcohol that is commonly used as a sugar substitute. It occurs naturally and is also produced synthetically from glucose. The food industry uses D-sorbitol as an additive in the form of a sweetener, humectant, emulsifier, thickener, or dietary supplement. D-Sorbitol has also been found in cosmetics, paper, and pharmaceuticals. Naturally, D-sorbitol occurs widely in plants via photosynthesis, ranging from algae to higher order fruits of the family *Rosaceae*.

In this assay, D-sorbitol is oxidized to fructose with the proportional development of intense color with an absorbance maximum at 560 nm. The assay is useful in the range of 0.1–10 nmoles of D-sorbitol per sample. This kit is suitable to measure D-sorbitol levels in samples such as foods, fruits, fruit juices, cosmetics, pharmaceuticals, and paper. The assay is NOT recommended for plasma, serum, or urine samples.

Components

The kit is sufficient for 100 assays in 96-well plates.

Sorbitol Assay Buffer Catalog Number MAK010A	25 mL
Sorbitol Probe Catalog Number MAK010B	0.2 mL
Sorbitol Enzyme Mix Catalog Number MAK010C	1 vial
Sorbitol Developer Catalog Number MAK010D	1 vial
Sorbitol Standard, 100 mM Catalog Number MAK010E	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96-well flat-bottom plate – It is recommended to use clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Sorbitol Enzyme Mix – Reconstitute vial in 220 µL of purified water. Mix well by pipetting, then aliquot and store, protected from light and moisture, at -20 °C.

Sorbitol Developer – Reconstitute vial in 1.0 mL of purified water. Mix well by pipetting. Keep on ice while in use. Aliquot and store protected from light and moisture at –20 °C.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

D-Sorbitol Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM Sorbitol Standard Solution with 990 µL of purified water to prepare a 1 mM standard solution. Mix well. Add 0, 2, 4, 8, 10 µL of the 1 mM Sorbitol standard solution into a 96-well plate, generating 0 (assay blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Sorbitol Assay Buffer to each well to bring the final volume to 50 µL.

Sample Preparation
Food product samples should be dissolved in purified water and centrifuged to remove insoluble material. Liquids such as juice should be diluted 10-fold (1:9) with purified water and centrifuged. Add 1–50 µL of sample to wells. Bring samples to a final volume of 50 µL with Sorbitol Assay Buffer.

Notes: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Interfering substances present in the sample can generate background. To control for background, include a blank sample for each sample by omitting the Enzyme Mix in the Reaction Mix.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Sample Blank	Samples and Standards
Sorbitol Assay Buffer	38 µL	36 µL
Sorbitol Enzyme Mix	–	2 µL
Sorbitol Developer	10 µL	10 µL
Sorbitol Probe	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.
3. Measure the absorbance at $A_{560\ nm}$ in kinetic mode at 37 °C. Read every 30 seconds for a total of 20 minutes, with gentle shaking for 2 seconds between measurements.

Results
Calculations
The background for the assay is the value obtained for the 0 (assay blank) Sorbitol standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Sorbitol standards to plot a standard curve.
Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of Sorbitol present in the sample may be determined from the standard curve.

Concentration of Sorbitol

$$S_a/S_v \times D = C \text{ (nmole/µL or mM)}$$

S_a = Amount of Sorbitol in unknown sample (nmole) from standard curve
S_v = Sample volume (µL) added into the well
D = Dilution factor, if necessary
C = Concentration of Sorbitol in sample

Sorbitol molecular weight: 182.17 g/mole

Sample Calculation
Amount of Sorbitol (S_a) = 2.4 nmole (from standard curve)
Sample volume (S_v) = 50 µL

Concentration of Sorbitol in sample

$$2.4 \text{ nmole}/50\ \mu\text{L} = 0.048 \text{ nmole}/\mu\text{L}$$

$$0.048 \text{ nmole}/\mu\text{L} \times 182.17 \text{ ng/nmole} = 8.74 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96-well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of improperly stored reagents	Check that components have been stored properly
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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