ab118968 – D-Sorbitol Assay Kit (Colorimetric)

For the measurement of sorbitol in a variety of samples.
For research use only - not intended for diagnostic use.
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab118968

Storage and Stability

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.

Materials Supplied

Item	Quantity	Storage Condition
Sorbitol Assay Buffer	25 mL	-20°C
Sorbitol Probe	0.2 mL	-20°C
Sorbitol Enzyme Mix/Sorbitol Enzyme Mix (Lyophilized)	1 vial	-20°C
Sorbitol Developer/Sorbitol Developer (Lyophilized)	1 vial	-20°C
Sorbitol Standard/Sorbitol Standard (100mM)	100 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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Reagent Preparation

– Before using the kit, spin the tubes prior to opening.

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.

Assay Protocol

Standard Curve Preparation:

1. 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.
2. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Sorbitol Standard.

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 analysed, 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.

Reaction Mix Preparation:

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

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

2. Add 50 µl of the Reaction Mix into each well.
3. Incubate for 30 min at 37 °C.

Measurement

Measure OD at 560 nm in a microplate reader with gentle shaking for 2 sec between measurements.

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:

$$\mathbf{C = S_a/S_v * D\ nmol/\mu l\ or\ mM}$$

Where: $\mathbf{S_a}$ = Sample amount (in nmol) from standard curve.
$\mathbf{S_v}$ = Sample volume (µl) added into the reaction wells.
D = Sample dilution factor if any.
D-Sorbitol MW: 182.17 g/mol.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)