RayBio® D-Sorbitol Colorimetric Assay Kit

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RayBio® Creatine Kinase Activity Colorimetric Assay Kit Protocol

(Cat#: 68CL-Dsorb-S100)

RayBiotech, Inc.

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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. RayBiotech'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. REAGENTS

Components	K777-100	Cap Code	Part Number
Sorbitol Assay Buffer	25	WM	Item A
Sorbitol Probe	200µl	Red	Item B
Sorbitol Enzyme Mix	Lyophilized	Green	Item C
Sorbitol Developer	Lyophilized	Blue	Item D
Sorbitol Standard (100 mM)	100ul	Yellow	Item E

III. STORAGE AND HANDLING

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 Procedure:

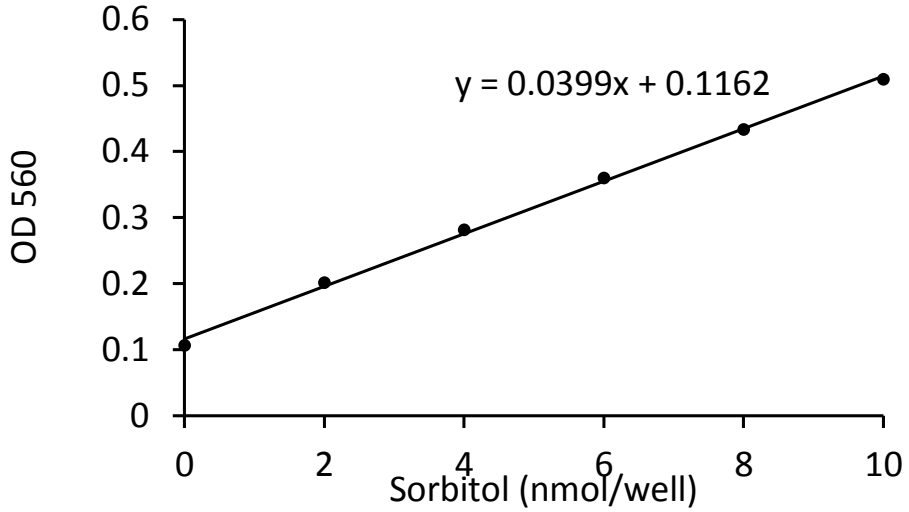
1. **Standard Curve Preparation:**
   Dilute the Sorbitol Standard to 1.0 mM by adding 10 µl of the Standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 96 well plate. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Sorbitol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

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

Where: $\mathbf{S_a}$ is the sample amount (in nmol) from standard curve.
$\mathbf{S_v}$ 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.



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