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Sucrose Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Sucrose levels in various samples

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

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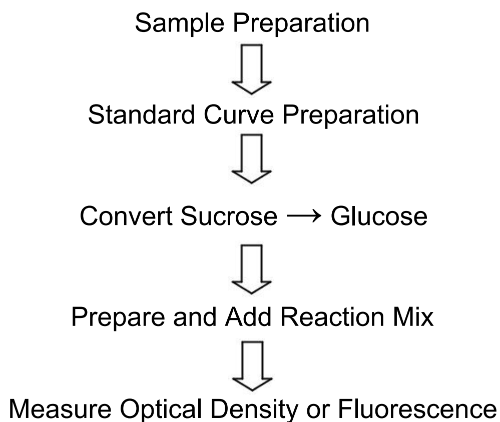
1. Overview

Sucrose ($C_{12}H_{22}O_{11}$; FW:342.3), also called table sugar, is one of the most important fuel sources to generate the universal energy molecule ATP. Sucrose is a disaccharide which can be converted into one glucose and one fructose.

Abcam's Sucrose Assay Kit provides a convenient means for specifically measuring sucrose levels from various biological samples (e.g. serum, plasma, body fluids, food, growth medium, etc.). Sucrose is converted to glucose and fructose by invertase. The generated glucose is then specifically oxidized to generate a product which reacts with a probe to generate color (λ= 570 nm) and fluorescence (Ex/Em = 535/587 nm). Use of proper controls allows for correcting free glucose background. Other disaccharides such as lactose and maltose do not interfere with the assay. The method can detect 0.0002-10 mM sucrose concentrations.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 2	25 mL
OxiRed™ Probe	0.2 mL
Invertase	1 vial
Developer Mix B	1 vial
Sucrose Standard	100 µL

* PLEASE NOTE: Developer Mix B was previously labelled as Development Enzyme Mix II and Enzyme Mix (Lyophilized), and Assay Buffer 2 as Assay Buffer II and Sucrose Assay Buffer. OxiRed™ Probe was previously labelled as OxiRed Probe and OxiRed Probe (in DMSO). The composition has not changed.
* Store kit at -20°C, protect from light. Allow buffers to warm to room temperature before use. Briefly centrifuge all small vials prior to opening. Keep enzymes on ice. Read the entire protocol before performing the assay.

OXIRED™ PROBE: Ready to use as supplied. Thaw the DMSO solution by warming to room temperature before use. Store at -20°C, protect from light and moisture. Use within two months.

INVERTASE AND DEVELOPER MIX B: Dissolve in 220 µl Assay Buffer 2 separately. Pipette up and down. Aliquot and store at -20 °C. Use within two months.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Prepare samples 1~50 µl in a 96-well plate, bring volume to a total of 50 µl with assay buffer. Serum can be directly diluted in the Assay Buffer 2.

For unknown samples, we suggest testing several doses to make sure the readings are within the standard curve linear range

2. Standard Curve Preparation:

a. For the colorimetric assay:

Dilute the Sucrose Standard solution to 1 mM (1 nmol/µl) by adding 10 µl of the 100 nmol/µl Sucrose Standard to 990 µl of Assay Buffer 2, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 50 µl/well with Assay Buffer 2 to generate 0, 2, 4, 6, 8, 10 nmol/well of the Sucrose Standard.

b. For the fluorometric assay:

Dilute 100 µl of the 1mM Sucrose Standard solution another 10 fold with 900 µl Assay Buffer 2 to 0.1 mM and mix well. Follow the same protocol as for the colorimetric assay. The fluorometric assay is ~10-100 fold more sensitive than the colorimetric assay.

3. Sucrose Conversion: Add 2 µl of Invertase* into sample and standard wells to convert sucrose to glucose.

* **Note:** Free Glucose interferes with the sucrose assay. If glucose is in your sample, you may do a glucose background control without the invertase. In the absence of invertase, the assay detects free glucose only, not the sucrose. Therefore, the free glucose background can be subtracted from the sample reading.

If the sample contains glucose, prepare two wells for each sample. To one well add 2 µl of Invertase to convert sucrose to glucose. To the other well, add 2µl of assay buffer without invertase as glucose background.

4. Reaction Mix: Mix enough reagents for the number of samples and standards to be measured. For each well, prepare a total 50 µl Reaction Mix containing:

Assay Buffer 2	46 µl
OxiRed™ Probe**	2 µl
Developer Mix B	2 µl

Mix well. Add 50 µl of the Reaction Mix to each well containing the Sucrose Standard or test samples. Mix well. Incubate the reaction for 30 min at 37°C, protect from light.

** **Note:** The fluorometric assay is 10 times more sensitive than the colorimetric assay. For detecting low concentrations of sucrose, use 0.4 µl of the sucrose probe per well in order to significantly decrease the fluorescence background. You must increase the amount of buffer to compensate for the decreased probe used.

5. Measurement: Read OD_{570nm} for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microplate reader.

5. Data Analysis

Subtract reagent background from all sample and standard assays.

Plot the standard curve. Correct background by subtracting the glucose background (without invertase) from all sample readings (with invertase). Apply sample OD to the standard curve.

Sucrose concentration can then be calculated:

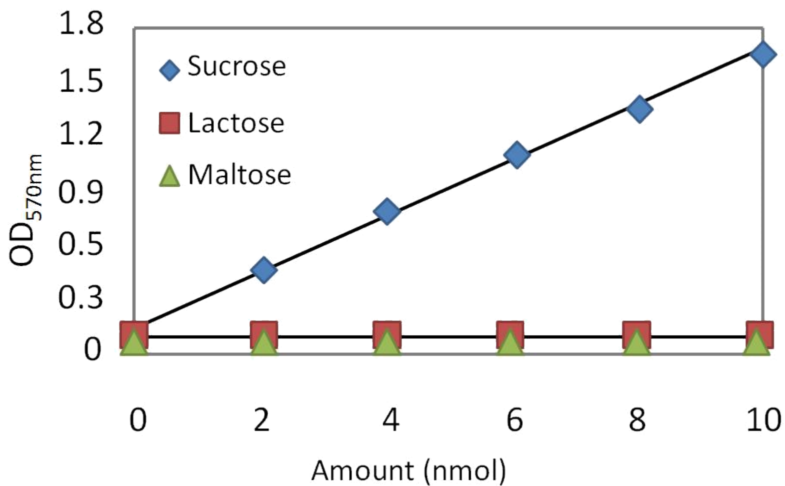
Concentration = Sa / Sv (nmol/µl or µmol/ml mM)

Where:

Sa is the sucrose amount from standard curve (nmol)

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

Sucrose Molecular Weight is 342.3



Sucrose Standard Curve. Different amounts of sucrose, lactose and maltose are assayed following the kit protocol.

Data show that the Sucrose assay kit specifically detects sucrose, but not lactose or maltose.

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).

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