BioVision

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For research use only

Sucrose Colorimetric/Fluorometric Assay Kit

(Catalog #K626-100; 100 assays; Store at -20˚C)

I. Introduction:

Sucrose ($C_{12}H_{22}O_{11}$; FW:342.3), also known as table sugar, is one of the most important fuel sources used to generate the universal energy molecule ATP. Sucrose is a disaccharide which is converted to one molecule of glucose and one molecule of fructose. BioVision'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 glucose is then specifically oxidized to generate a product which reacts with a probe to generate color (λ= 570 nm) and fluorescence (Ex/Em = 535/587nm). Use of proper controls allows for correcting for the 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.

II. Kit Contents

Component	K626-100	Cap Code	Parts Number
Sucrose Assay Buffer	25 ml	WM	K626-100-1
OxiRed Probe (in DMSO)	0.2 ml	Red	K626-100-2A
Invertase (Lyophilized)	1 Vial	Blue	K626-100-4
Enzyme Mix (Lyophilized)	1 Vial	Green	K626-100-5
Sucrose Standard (100 nmol/µl)	100 µl	Yellow	K626-100-6

III. Storage and Handling:

Store kit at –20˚C, protect from light. Briefly centrifuge vials prior to opening. Allow buffers warm to room temperature before use. Keep enzymes on ice.

IV. Reagent Preparation:

OxiRed Probe: Ready to use as supplied. Warm to 18°C (~ room temperature) before use to melt frozen DMSO. Store at –20˚C, protect from light and moisture. Use within two months.

Invertase: Dissolve in 220 µl Sucrose Assay Buffer, pipette up and down. Aliquot and store at –20˚C. Use within two months.

Enzyme Mix: Dissolve in 220 µl Sucrose Assay Buffer. Pipette up and down. Aliquot and store at –20˚C. Use within two months.

V. Assay Protocol:

1. Standard Curve Preparation:

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

For the fluorometric assay, dilute100 µl of the 1mM Sucrose Standard solution another 10 fold with900 µl Assay Buffer 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.

2. **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. For unknown samples, we suggest testing several dosesto make sure the readings are within the standard curve linear range.

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

Glucose Background: 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. **Assay 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:

46 µl Assay Buffer
2 µl OxiRed Probe*
2 µl Enzyme Mix

Mix well. Add 50 µl of the Reaction Mix to each well containing the Sucrose Standard ortest 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. Measure OD $_{570nm}$ for the colorimetric assay or Ex/Em = 535/590 nm for the fluorometric assay in a microplate reader.

6. **Calculations:** 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:

C = Sa / Sv nmol/µl or µmol/ml or mM

Where: **Sa** is sucrose amount from standard curve (nmol).
Sv is the sample volume added to sample wells (µl).
Sucrose Molecular Weight 342.3.

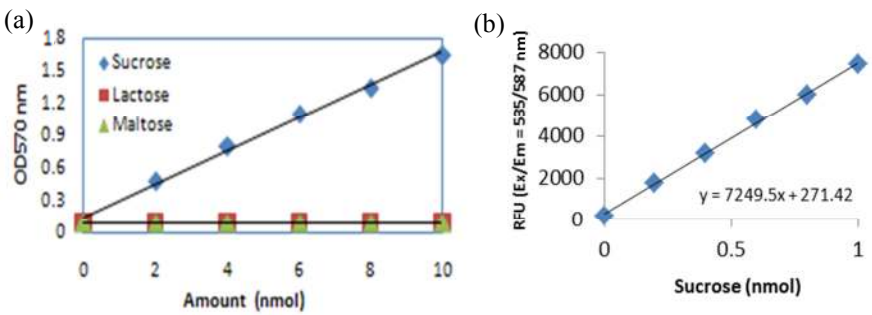

Figure: Sucrose Standard Curve: (a) Different amounts of sucrose, lactose and maltose are assayed following the kit protocol. Data show that the Sucrose assay kit specifically detects sucrose, but not lactose or maltose. (b) Fluorometric Standard Curve

RELATED PRODUCTS:

Glucose Assay Kit
Galactose Assay Kit
Lactose Assay Kit
Pyruvate Assay Kit
Cell Fractionation Kits
Fructose Assay Kit
Maltose Assay Kit
Lactate Assay Kit
Apoptosis Assay Kits
Cell Proliferation Assays

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• 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 an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		