Glucose and Sucrose Cc

(Catalog #K616-100; 100 assays; Store at -20°C)

I. Introduction:

Glucose ($C_6H_{12}O_6$; FW: 180.16) and sucrose ($C_{12}H_{22}O_{11}$; FW: 342.3) are the important fuel sources to generate the universal energy molecule ATP. Measurement of glucose or sucrose levels can be very important in both research and development processes. Sucrose is a disaccharide which can be cleaved into one glucose and one fructose molecule using Invertase. BioVision's Glucose and Sucrose Assay Kit provides a convenient means for measuring glucose and sucrose levels from various biological samples (e.g. serum, plasma, body fluids, food, growth media, etc.). To measure glucose level, glucose oxidase specifically oxidizes free glucose generating a compound that reacts with the glucose probe to produce resorufin, which can be detected colorimetrically (O.D. 570 nm) or fluorometrically (Ex/Em 535/587). To measure sucrose, invertase is added to the reaction to convert sucrose to free glucose and fructose, so total glucose level can be measured. Then the sucrose level = Total Glucose – Free Glucose.

II. Kit Contents

Component	K616-100	Cap Code	Part Number
Glucose Assay Buffer	25 ml	WM	K616-100-1
Glucose Probe (in DMSO)	200µl	Red	K616-100-2A
Invertase (Lyophilized)	1 Vial	Blue	K616-100-4
Glucose Enzyme Mix (Lyophilized)	1 Vial	Green	K616-100-5
Sucrose Standard (100 nmol/µl)	100 µl	Yellow	K616-100-6

III. Storage and Handling:

Store kit at –20˚C, protect from light. Allow reagents warm to room temperature and briefly centrifuge vials before opening.

IV. Reagent Preparation:

Glucose Probe: Ready to use as supplied. Warm to room temperature prior to use to melt frozen DMSO. Store at –20°C, protect from light and moisture. Use within two months.

Invertase, Glucose Enzyme Mix: Dissolve in 220 µl Glucose Assay Buffer. Aliquot and store at –20°C. Use within two months.

V. Assay Protocol:

1. Standard Curve Preparations:

For colorimetric assay, dilute the Sucrose Standard to 1 nmol/µl by adding 10 µl of the Sucrose Standard to 990 µl of Glucose Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Glucose Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Sucrose Standard.

For fluorometric assay, dilute the Sucrose Standard solution to 0.1 nmol/µl by adding 10 µl of the Sucrose Standard to 990 µl of Glucose Assay Buffer, mix well. Then take 20 µl into 180 µl of Glucose Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually.

Adjust volume to 50 µl/well with Glucose Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Sucrose Standard. Fluorometric assay is 10-100 fold more sensitive than colorimetric assay.

2. Sample Preparations:

Prepare test samples in 50 µl/well with Glucose Assay Buffer in a 96-well plate. Serum can be directly diluted in the Glucose Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve linear range. For Sucrose detection, prepare two wells for each sample. To one well, add 2 µl of Invertase to convert sucrose to glucose by incubating the invertase reaction at 37˚C for 30 min before adding Glucose Assay Mix in next step. To the other vial, omit Invertase the assay detects free glucose only. Sucrose = Total Glucose – Free Glucose.

Note: 2 µl of invertase must be added to each well of the Sucrose Standard to convert sucrose standard to glucose for either glucose or sucrose assay.

3. Glucose Assay Mix:

Mix enough reagent for the total number of assays (samples + standards) to be performed.

For each well, prepare a total 50 µl Reaction Mix containing:

46 µl Glucose Assay Buffer
2 µl Glucose Probe
2 µl Glucose Enzyme Mix

4. 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 minutes at 37˚C, protect from light.

5. Measure OD $_{570nm}$ for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microplate reader.

6. Correct background by subtracting the value derived from the 0 sucrose control from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Glucose concentrations of the test samples can then be calculated based on the standard curve you generated, the dilution factor, and volume of your samples added into the wells. Sucrose = Total Glucose – Free Glucose.

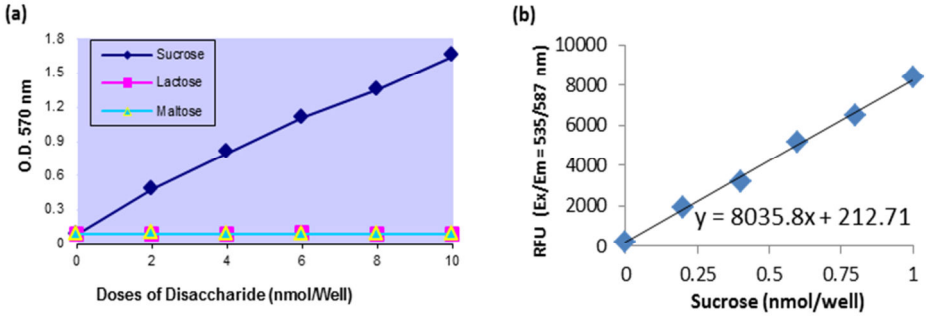

Figure: Sucrose Standard Curve: (a) Colorimetric (b) Fluorometric. Standard Curves were generated following kit protocol.

RELATED PRODUCTS:

- Lactate Assay Kit
- Galactose, Lactose Assay Kit
- Glucose, Maltose Assay Kit
- NAD/NADH Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

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.		