

Glucose and Sucrose Assay Kit

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

I. Introduction:

Glucose (C₆H₁₂O₆; FW: 180.16) and sucrose (C₁₂H₂₂O₁₁; FW:342.3) are the important fuel sources to generate universal energy molecule ATP. Measurement of glucose or sucrose level can be very important in both research and development process. Sucrose is a disaccharide which can be converted into one glucose and one fructose when adding 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 medium, 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 can be 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	Parts #
Glucose Assay Buffer	25 ml	WM	K616-100-1
Glucose Probe (Lyophilized)	1 Vial	Red	K616-100-2
Dimethylsulfoxide (DMSO; H ₂ O-free)	0.4 ml	Brown	K616-100-3
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: Dissolve in 220 μl DMSO (provided) before use. Store at -20°C, protect from light and moisture. Use within two months.

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

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.

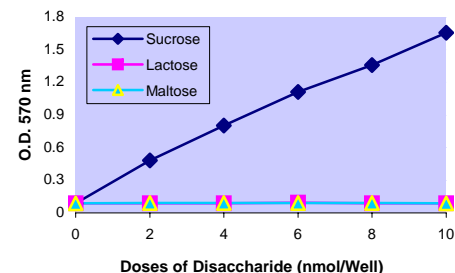
- Sample Preparations:** Prepare test samples in 50 μl/well with Glucose Assay Buffer in a 96-well plate. Serum can be directly diluted in the Glucose Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve linear range. For Sucrose detection, 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.

- Glucose Assay Mix:** Mix enough reagents for the number of assays and standard 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

- 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.
- Measure O.D. _{570nm} for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microplate reader.
- 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.



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