Glucose Assay Kit

(Catalog #K606-100; 100 assays; Store at -20 °C.)

I. Introduction:

Glucose ($C_6H_{12}O_6$; FW: 180.16) is a very important fuel source to generate the universal energy molecule ATP. Glucose level is a key diagnostic parameter for many metabolic disorders. Measurement of glucose can be very important in both research and drug discovery processes. The BioVision Glucose Assay Kit provides direct measurement of glucose in various biological samples (e.g., serum, plasma, other body fluids, food, growth media, etc.). Glucose Enzyme Mix specifically oxidizes glucose to generate a product which reacts with a dye to generate color (λ = 570 nm) and fluorescence (Ex/Em = 535/587 nm). The color and fluorescence generated is proportional to the amount of glucose present. The method is rapid, simple, sensitive, and suitable for high throughput. The assay is also suitable for monitoring glucose level during fermentation and glucose feeding in protein expression processes. The kit detects 1-10000 µM glucose samples.

II. Kit Contents

Component	K606-100	Cap Code	Part Number
Glucose Assay Buffer	25 ml	WM	K606-100-1
Glucose Probe (in DMSO)	0.2 ml	Red	K606-100-2A
Glucose Enzyme Mix (lyophilized)	1 vial	Green	K606-100-4
Glucose Standard (100 nmol/µl)	100 µl	Yellow	K606-100-5

III. Storage and Handling:

Store kit at –20˚C, protect from light. Warm the Glucose Assay Buffer to room temperature and briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Preparation:

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

Glucose Enzyme Mix: Dissolve in 220 µl Glucose Assay Buffer, then aliquot & store at –20 ˚C. Keep on ice while in use. Use within two months.

V. Glucose Assay Protocol:

1. Standard Curve Preparations:

For colorimetric assay,dilute the Glucose Standard to 1 nmol/µl by adding 10 µl of the Glucose Standard to 990 µl of Glucose Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of well on a 96 well plate. Adjust volume to 50 µl/well with Glucose Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Glucose Standard.

For the fluorometric assay, dilute the Glucose Standard solution to 0.1 nmol/µl by adding 10 µl of the Glucose Standard to 990 µl of Glucose Assay Buffer, mix well. Then take 20 µl into180 µl of Glucose Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells as in the colorimetric assay. 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 Glucose Standard.

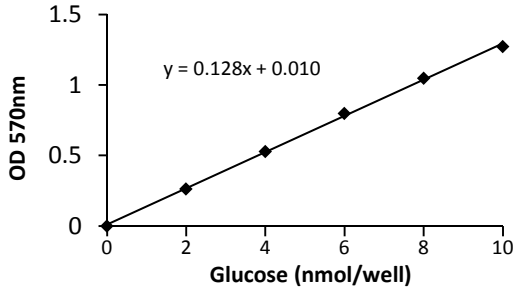
2. **Sample Preparation:** Prepare test samples at a total volume of 50 µl/well with Glucose Assay Buffer in a 96-well plate. If using serum, limit sample volume to 0.5-2 µl/assay. Adjust the final volume to 50 µl with Assay buffer. Normal serum contains ~5 nmol/µl glucose can be directly diluted in the Glucose Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.
3. **Glucose Reaction Mix:** Mix enough reagent for the number of assays 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

   ***Note:** The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µl of the probe per reaction to decrease the background reading / increase detection sensitivity significantly.
4. Mix well. Add 50 µl of the Reaction Mix to each well containing the Glucose Standard and test samples. Mix well.
5. Incubate the reaction for 30 minutes at 37 ˚C, protect from light. Measure absorbance at 570 nm for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microplate reader.
6. **Calculations:** Correct background by subtracting the value derived from the 0 glucose control from all 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.

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

   Where Sa is sample amount (in nmol) from standard curve.
   Sv is sample volume (in µl) added into the sample wells.
   Glucose Molecular Weight 180.16.



VI. RELATED PRODUCTS:

Glutathione Assay Kit
Cell Proliferation & Senescence
Cholesterol and Obesity Assay Kits
Galactose Assay Kit
Lactate Assay Kit
Cell Fractionation Kits
Cell Damage & Stress Products
Sucrose Assay Kit
Ethanol Assay Kit
Pyruvate Assay Kit

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.		

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