Glucose Colorimetric Assay Kit II

(Catalog # K686-100; 100 assays; Store at -20°C)

I. Introduction:

Glucose is an important fuel source to generate the universal energy molecule, ATP. Serum glucose is a key diagnostic parameter for many metabolic disorders. BioVision's Glucose Assay Kit II provides direct measurement of glucose in various biological samples (e.g., serum, plasma, other body fluids, food, growth media, etc.). In this assay, glucose is specifically oxidized to generate a product which reacts with a dye to generate color (λ = 450 nm) whose intensity is proportional to glucose concentration. The method is rapid, simple, sensitive, and suitable for high throughput applications. This assay is particularly suitable for serum and urine samples since it is unaffected by reducing substances which can interfere with other suppliers offering oxidase-based kits. The assay is also suitable for monitoring glucose level during fermentation and glucose feeding in protein expression processes. The kit can detect glucose concentrations in the range of 20 µM – 10 mM.

II. Kit Contents:

Components	K686-100	Cap Color	Part Number
Glucose Assay Buffer	25 ml	WM	K686-100-1
Glucose Substrate Mix (lyophilized)	1 vial	Red	K686-100-2
Glucose Enzyme Mix (lyophilized)	1 vial	Green	K686-100-3
Glucose Standard (100 mM)	100 µl	Yellow	K686-100-4

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 Substrate Mix: Dissolve in 220 µl Glucose Assay Buffer. Aliquot and store at -20°C, protect from light and moisture. Use within two months.

Glucose Enzyme Mix: Dissolve in 220 µl dH_2O. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

V. Glucose Assay Protocol:

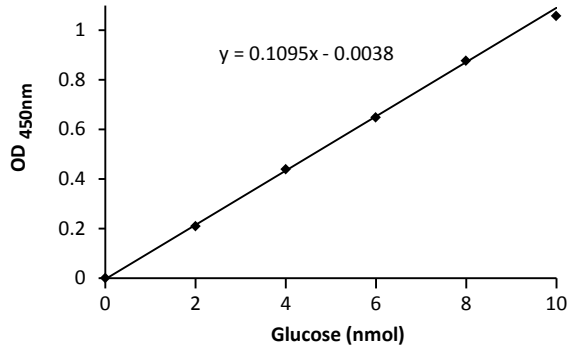
1. **Standard Curve Preparations:**
   Dilute the Glucose Standard to 1 nmol/µl by adding 10 µl of the Glucose Standard to 990 µl of Glucose Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells of a 96 well plate. Adjust volume of all wells to 50 µl with Glucose Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Glucose Standard.
2. **Sample Preparations:** Prepare test samples in a total volume of 50 µl/well with Glucose Assay Buffer in a 96-well plate. If using serum sample, serum (0.5 - 2 µl/assay. Normal serum contains ~5 nmol/µl glucose) can be directly diluted in the Glucose Assay Buffer. We recommend deproteinizing samples by centrifugation using a 10 kDa spin column (Cat#1997-25) to remove enzymes and interfering proteins. For unknown samples, 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 reagents 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 Enzyme Mix
   2 µl Glucose Substrate Mix
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 min at RT, protect from light.
6. Measure absorbance at 450 nm in a microplate reader.
7. **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). Plot the standard curve. Apply the sample readings to the standard curve. 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) calculated from standard curve.
   Sv is sample volume (in µl) added into the sample wells.
   (Note: if sample was pre-diluted before added to reaction well-must correct for this dilution factor)

Glucose Molecular Weight 180.16
Normal serum glucose range: 3 - 7 mM, Normal urine glucose range: 0 - 0.8 mM



Standard Curve for Glucose Run Using the Kit Protocol

RELATED PRODUCTS:

Glutathione Assay Kit
Cell Proliferation & Senescence
Cholesterol and Obesity Assay Kits
Sucrose Assay Kit
Galactose Assay Kit
Lactate Assay Kit
Cell Fractionation Kits
Cell Damage & Stress Products
Glucose Colorimetric/Fluorometric Assay
Fatty Assay Kit
Ethanol Assay Kit
Pyruvate 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.		