Product Information

Glucose Colorimetric Assay Kit II

I. Kit Contents:

Components	K2219-100 100 assays	Cap Color	Part Number
Glucose Assay Buffer	25 ml	WM	K2219-C-1
Glucose Substrate Mix (lyophilized)	1 vial	Red	K2219-C-2
Glucose Enzyme Mix (lyophilized)	1 vial	Green	K2219-C-3
Glucose Standard (100 mM)	100 µl	Yellow	K2219-C-4

II. Introduction:

Glucose ($C_6H_{12}O_6$) is an important fuel source to produce energy molecule ATP. Serum glucose level is a key diagnostic parameter for many metabolic disorders. Measurement of glucose level is very important in both drug discovery and research processes.

The Glucose Colorimetric Assay Kit II provides a sensitive, simple, fast and convenient way for detection of glucose levels in various biological samples (plasma, serum, other body fluids, growth media, food, etc.) based on colorimetric method. In the assay, glucose is specifically oxidized to yield a product which reacts with a dye to generate color (λ = 450 nm). The color generated is proportionally to glucose concentration. The assay is particularly suited for urine and serum samples since it is unaffected by reducing substances in samples. The assay is suited for high throughput screening and is also suited for monitoring glucose feeding in protein expression processes and glucose level during fermentation. The kit can detect 20 µM - 10 mM glucose concentrations.

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 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:

Glucose Assay Buffer 46 µl

Glucose Enzyme Mix 2 µl

Glucose Substrate Mix 2 µl

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, 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)

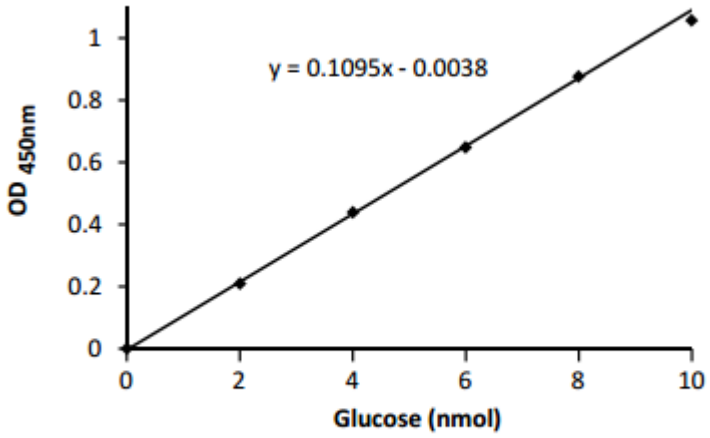
Where: Sa is sample amount (in nmol) calculated from standard curve.

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

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet)	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as

	• Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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