



## Glucose Assay Kit

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

### I. Introduction:

Glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>; 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 ( $\lambda = 570$  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  $\mu$ M 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/ul)	100 ul	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  $\mu$ l 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/ $\mu$ l by adding 10  $\mu$ l of the Glucose Standard to 990  $\mu$ l of Glucose Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10  $\mu$ l into a series of well on a 96 well plate. Adjust volume to 50  $\mu$ l/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/ $\mu$ l by adding 10  $\mu$ l of the Glucose Standard to 990  $\mu$ l of Glucose Assay Buffer, mix well. Then take 20  $\mu$ l into 180  $\mu$ l of Glucose Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10  $\mu$ l into a series of wells as in the colorimetric assay. Adjust volume to 50  $\mu$ l/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  $\mu$ l/well with Glucose Assay Buffer in a 96-well plate. If using serum, limit sample volume to 0.5-2  $\mu$ l/assay. Adjust the final volume to 50  $\mu$ l with Assay buffer. Normal serum contains ~5 nmol/ $\mu$ l 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  $\mu$ l Reaction Mix containing:  
46  $\mu$ l Glucose Assay Buffer  
2  $\mu$ l Glucose Probe\*  
2  $\mu$ l Glucose Enzyme Mix

**\*Note:** The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4  $\mu$ l of the probe per reaction to decrease the background reading / increase detection sensitivity significantly.

4. Mix well. Add 50  $\mu$ l 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  $^{\circ}$ 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.

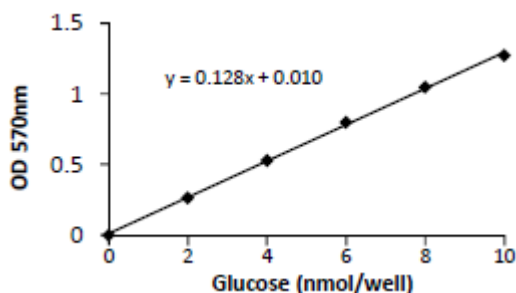
**6. Calculations:** Correct background by subtracting the value derived from the 0 glucose control from all readings (Note: The background reading can be significant and must be subtracted from sample readings). Glucose concentrations of the test samples can then be calculated.

$$C = Sa/Sv \text{ (nmol/}\mu\text{l or } \mu\text{mol/ml, or mM)}$$

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

Sv is sample volume (in  $\mu$ l) 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

## GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"><li>• Use of ice-cold assay buffer</li><li>• Omission of a step in the protocol</li><li>• Plate read at incorrect wavelength</li><li>• Use of a different 96-well plate</li></ul>	<ul style="list-style-type: none"><li>• Assay buffer must be at room temperature</li><li>• Refer and follow the data sheet precisely</li><li>• Check the wavelength in the data sheet and the filter settings of the instrument</li><li>• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates</li></ul>
Samples with erratic readings	<ul style="list-style-type: none"><li>• Use of an incompatible sample type</li><li>• Samples prepared in a different buffer</li><li>• Samples were not deproteinized (if indicated in datasheet)</li><li>• Cell/ tissue samples were not completely homogenized</li><li>• Samples used after multiple free-thaw cycles</li><li>• Presence of interfering substance in the sample</li><li>• Use of old or inappropriately stored samples</li></ul>	<ul style="list-style-type: none"><li>• Refer data sheet for details about incompatible samples</li><li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li><li>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated</li><li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li><li>• Aliquot and freeze samples if needed to use multiple times</li><li>• Troubleshoot if needed, deproteinize samples</li><li>• Use fresh samples or store at correct temperatures till use</li></ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"><li>• Improperly thawed components</li><li>• Use of expired kit or improperly stored reagents</li><li>• Allowing the reagents to sit for extended times on ice</li><li>• Incorrect incubation times or temperatures</li><li>• Incorrect volumes used</li></ul>	<ul style="list-style-type: none"><li>• Thaw all components completely and mix gently before use</li><li>• Always check the expiry date and store the components appropriately</li><li>• Always thaw and prepare fresh reaction mix before use</li><li>• Refer datasheet &amp; verify correct incubation times and temperatures</li><li>• Use calibrated pipettes and aliquot correctly</li></ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"><li>• Use of partially thawed components</li><li>• Pipetting errors in the standard</li><li>• Pipetting errors in the reaction mix</li><li>• Air bubbles formed in well</li><li>• Standard stock is at an incorrect concentration</li><li>• Calculation errors</li><li>• Substituting reagents from older kits/ lots</li></ul>	<ul style="list-style-type: none"><li>• Thaw and resuspend all components before preparing the reaction mix</li><li>• Avoid pipetting small volumes</li><li>• Prepare a master reaction mix whenever possible</li><li>• Pipette gently against the wall of the tubes</li><li>• Always refer the dilutions in the data sheet</li><li>• Recheck calculations after referring the data sheet</li><li>• Use fresh components from the same kit</li></ul>
Unanticipated results	<ul style="list-style-type: none"><li>• Measured at incorrect wavelength</li><li>• Samples contain interfering substances</li><li>• Use of incompatible sample type</li><li>• Sample readings above/below the linear range</li></ul>	<ul style="list-style-type: none"><li>• Check the equipment and the filter setting</li><li>• Troubleshoot if it interferes with the kit</li><li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li><li>• Concentrate/ Dilute sample so as to be in the linear range</li></ul>

Note# The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.

GENT