

# Glucose Assay Kit II

High-throughput adaptable colorimetric assay to determine glucose in various biological samples

## Instruction Manual

Catalog Number	PK-CA577-K686															
Description	<p>Glucose is an important fuel source to generate the universal energy molecule, ATP. Serum glucose is a key diagnostic parameter for many metabolic disorders.</p> <p>PromoKine'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 (<math>\lambda = 450</math> 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 <math>\mu</math>M – 10 mM.</p>															
Quantity	100 assays															
Applications	<ul style="list-style-type: none"><li>• Measurement of glucose in various samples</li><li>• Analysis of carbohydrate metabolism</li></ul>															
Sample Type	<ul style="list-style-type: none"><li>• Serum, plasma, urine &amp; other body fluids</li><li>• Growth media</li><li>• Food</li></ul>															
	<table border="1"><thead><tr><th>Components</th><th>Quantity</th><th>Color Code</th></tr></thead><tbody><tr><td>Glucose Assay Buffer</td><td>25 ml</td><td>WM</td></tr><tr><td>Glucose Substrate Mix (lyophilized)</td><td>1 vial</td><td>Red</td></tr><tr><td>Glucose Standard (100 mM)</td><td>100 <math>\mu</math>l</td><td>Yellow</td></tr><tr><td>Glucose Enzyme Mix (lyophilized)</td><td>1 vial</td><td>Green</td></tr></tbody></table>	Components	Quantity	Color Code	Glucose Assay Buffer	25 ml	WM	Glucose Substrate Mix (lyophilized)	1 vial	Red	Glucose Standard (100 mM)	100 $\mu$ l	Yellow	Glucose Enzyme Mix (lyophilized)	1 vial	Green
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User Supplied Reagents & Equipment	<ul style="list-style-type: none"><li>• 96-well plate with clear flat bottom</li><li>• Multi-well spectrophotometer</li></ul>															
Storage and Reagents Preparation	<p>Store kit at <math>-20^{\circ}\text{C}</math>, protected from light. Warm the Glucose Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.</p> <ul style="list-style-type: none"><li>• <b>Glucose Substrate Mix:</b> Dissolve in 220 <math>\mu</math>l Glucose Assay Buffer. Aliquot and store at <math>-20^{\circ}\text{C}</math>, protect from light and moisture. Use within two months.</li><li>• <b>Glucose Enzyme Mix:</b> Dissolve in 220 <math>\mu</math>l dH<sub>2</sub>O. Aliquot and store at <math>-20^{\circ}\text{C}</math>. Keep on ice while in use. Use within two months.</li></ul>															
Assay Protocol	<p><b>1. Standard Curve Preparations:</b></p> <p>Dilute the Glucose Standard to 1 nmol/<math>\mu</math>l by adding 10 <math>\mu</math>l of the Glucose Standard to 990 <math>\mu</math>l of Glucose Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 <math>\mu</math>l into a series of wells of a 96 well plate. Adjust volume of all wells to 50 <math>\mu</math>l with Glucose Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Glucose Standard.</p> <p><b>2. Sample Preparations:</b> Prepare test samples in a total volume of 50 <math>\mu</math>l/well with Glucose Assay Buffer in a 96-well plate. If using serum sample, serum (0.5 - 2 <math>\mu</math>l/assay. Normal serum contains ~5 nmol/<math>\mu</math>l glucose) can be directly diluted in the Glucose Assay Buffer. We recommend deproteinizing samples by centrifugation using a 10 kDa spin column (Cat.No. PK-CA577-1997) 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.</p> <p><b>3. Glucose Reaction Mix:</b> Mix enough reagents for the number of assays to be performed: For each well, prepare a total 50 <math>\mu</math>l Reaction Mix containing:</p> <ul style="list-style-type: none"><li>46 <math>\mu</math>l Glucose Assay Buffer</li><li>2 <math>\mu</math>l Glucose Enzyme Mix</li><li>2 <math>\mu</math>l Glucose Substrate Mix</li></ul>															

Mix well. Add 50  $\mu\text{l}$  of the Reaction Mix to each well containing the Glucose Standard and test samples. Mix well. Incubate the reaction for 30 minutes at RT, protect from light.

**4. Measurement:** Measure absorbance at 450 nm in a microplate reader.

**5. 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/ $\mu\text{l}$  or  $\mu\text{mol}/\text{ml}$ , or mM)**

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

Sv is sample volume (in  $\mu\text{l}$ ) 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

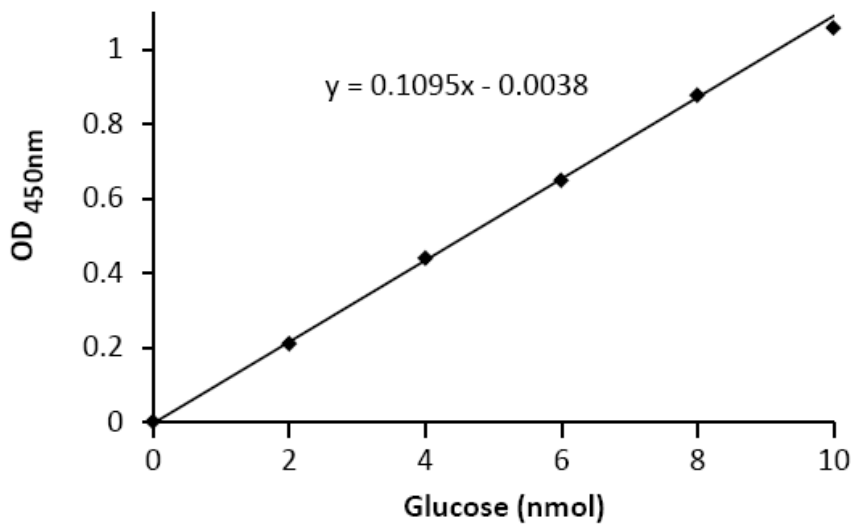


Figure: Standard Curve for Glucose Run using the kit protocol.

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