ab102517

Glucose Detection Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Glucose levels in various samples

View kit datasheet: www.abcam.com/ab102517
(use www.abcam.cn/ab102517 for China, or www.abcam.co.jp/ab102517 for Japan)

This product is for research use only and is not intended for diagnostic use.

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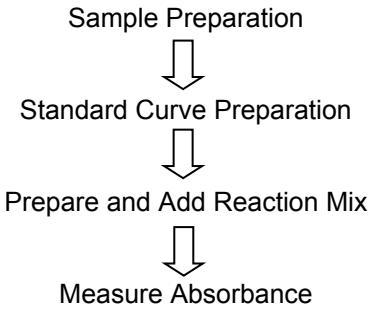
1. Overview

Glucose is an important fuel source to generate the universal energy molecule ATP. Serum glucose level is a key diagnostic parameter for many metabolic disorders.

Abcam's Glucose Detection Kit 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. 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 20µM-10mM.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Glucose Assay Buffer	25 mL
Glucose Substrate Mix (Lyophilized)	1 vial
Glucose Enzyme Mix (Lyophilized)	1 vial
Glucose Standard (100 mM)	100 µL

* Store kit at -20°C, protect from light.

- Allow Assay Buffer to warm to room temperature before use.
- Briefly centrifuge all small vials prior to opening.
- Read the entire protocol before performing the assay.

GLUCOSE SUBSTRATE MIX AND ENZYME MIX: Dissolve separately in 220 µl Glucose Assay Buffer. Aliquot and store at -20°C, protect from light and moisture. Use within two months.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Prepare test samples in 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.

Note:

It is recommended to de-proteinize samples by centrifugation using a 10 kDa spin column (ab93349) 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.

2. Standard Curve Preparation:

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.

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:

Glucose Assay Buffer	46 µl
Glucose Enzyme Mix	2 µl
Glucose Substrate Mix	2 µl

Mix well. Add 50 µl of the Reaction Mix to each well containing the Glucose Standard and test samples, mix well. Incubate the reaction for 30 min, protect from light.

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

5. Data Analysis

Correct background by subtracting the value derived from the zero glucose control from all readings. 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:

Concentration = Sa / Sv (nmol/µl or mM)

Where:

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

Sv is sample volume (µl) added to the wells.

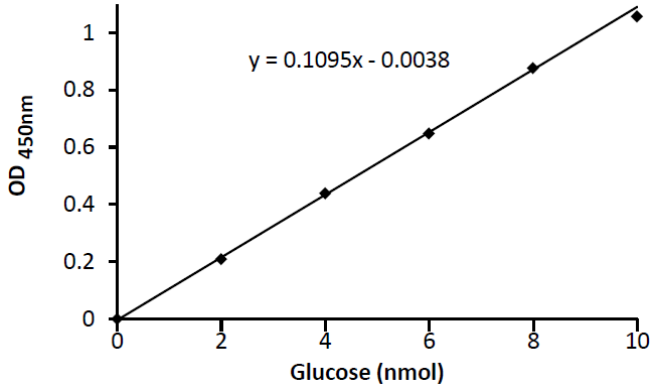
Note:

If sample was pre-diluted before added to reaction well you 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 carried out using the Kit Protocol

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

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