ab169559 - Glucose Assay Kit (Fluorometric, High Sensitivity)

Instructions for Use

For the accurate measurement of glucose in cells and tissues, plasma, serum and other body fluids, growth media and food.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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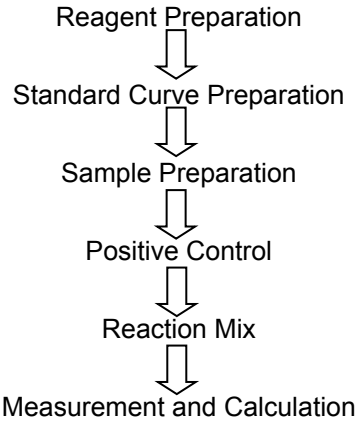
1. Overview

Glucose is the main energy source for virtually all living organisms. 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.

Abcam's Glucose Assay Kit (Fluorometric, High Sensitivity) (ab169559) is simple, rapid, ultra-sensitive and suitable for high-throughput use. In this assay, D-glucose is enzymatically oxidized to form a product which reacts with a colorless probe to generate the fluorescence (Ex/Em = 535/587nm). The fluorescence generated is directly proportional to the amount of glucose. This assay kit can detect less than 0.5 µM glucose in various biological samples.

2. Protocol Summary



3. Kit Components

Item	Quantity	Storage upon arrival	Storage after use/ reconstitution
Assay Buffer II/Glucose Assay Buffer	25 mL	-20°C	-20°C
PicoProbe I/PicoProbe™ Probe (in DMSO)	0.4 mL	-20°C	-20°C
Glucose Enzyme Mix/Glucose Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
Substrate Mix I/Glucose Substrate Mix (lyophilized)	1 vial	-20°C	-20°C
Glucose Standard/Glucose Standard (100 mM)	100 µL	-20°C	-20°C

4. Storage

Store the kit at -20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm assay buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

5. Additional Materials Required

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well spectrophotometer (ELISA reader)

6. Assay Protocol

A. Reagent Preparation

1. PicoProbe I/PicoProbe™:

Ready to use as supplied. Warm to room temperature before use. Store at -20°C.

2. Glucose Enzyme Mix:

Reconstitute with 220 µL Assay Buffer II/Glucose Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Stable for 2 months at -20°C.

3. Substrate Mix I/Glucose Substrate Mix:

Dissolve with 220 µL dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Stable for 2 months at -20°C.

B. Glucose Assay Protocol

1. Glucose Standard Curve:

Dilute Glucose Standard to 1 mM by adding 10 µl of Glucose Standard/100 mM Glucose Standard to 990 µl dH_2O, mix well. Dilute 1 mM Glucose Standard further to 10 µM (10 pmol/µl) by adding 10 µl of 1 mM Glucose Standard to 990 µl of dH_2O. Mix well. Add 0, 2, 4, 6, 8 & 10 µl of 10 µM Glucose Standard into series of wells in 96 well plate to generate 0, 20, 40, 60, 80 and 100 pmol/well of Glucose Standard. Adjust volume to 50 µl/well with Assay Buffer II/Glucose Assay Buffer.

2. Sample preparation:

Liquid samples can be measured directly. Tissue (10 mg) or cells (1×10^6) should be homogenized on ice with 100 µL ice cold Assay Buffer II/Glucose Assay Buffer. Centrifuge at 12,000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (1-10 µg) into a 96 well plate and adjust the volume to 50 µL with Assay Buffer II/Glucose Assay Buffer.

Notes: Protein and various enzymes in samples may interfere with the assay, we recommend deproteinizing the samples using either a perchloric acid/KOH protocol or by spin filtering through a 10kD membrane.

For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.

NADH in samples will generate background. For samples having high NADH levels, a sample background control may be required.

3. Reaction Mix:

Mix enough reagents for the number of samples and standards to be performed. For each well, prepare a total 50 µL Reaction Mix containing:

	Reaction Mix	Background Control Mix
Assay Buffer II/Glucose Assay Buffer	45 µL	47 µL
PicoProbe I/PicoProbe™	1 µL	1 µL
Glucose Enzyme Mix	2 µL	--
Substrate Mix I/Glucose Substrate Mix	2 µL	2 µL

Add 50 µL of the Reaction Mix to each well containing the standard and test samples, mix well.

Note: For samples having high NADH levels, add 50 µL of Background Control Mix to sample background control well(s). Mix well.

4. Measurement

Incubate the reaction for 30 minutes at 37°C, protected from light. Measure fluorescence at Ex/Em = 535/587 nm in a micro plate reader.

7. Data Analysis

Calculation: Subtract 0 Glucose Standard reading from all readings. Plot the Glucose Standard curve. If sample background control reading is significantly high, subtract the background control reading from sample reading. Apply the corrected sample reading to the Glucose Standard curve to get B pmol of Glucose in the sample wells.

$$\text{Sample glucose concentration} = \frac{B}{V} \times \text{Dilution Factor} = \text{pmol/µL} = \text{nmol/mL or µM}$$

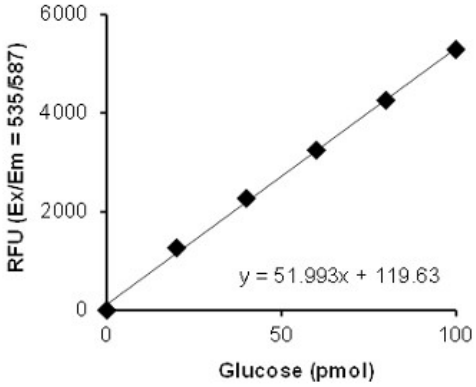
Where:

B amount of glucose in the sample from Standard curve (pmol)

V sample volume added in the reaction well (µL)

Glucose in sample can also be expressed in nmol/mg of sample. Glucose molecular weight: 180.2 g/mol.

(a)



(b)

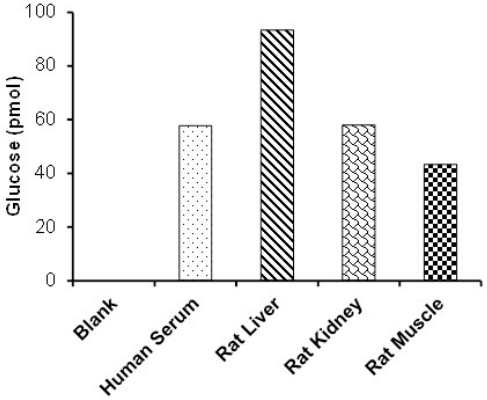


Figure 1: (a) Glucose standard curve (b) Measurement of glucose levels in human serum (1 µL of 1:10 diluted) and rat tissue lysates from liver, kidney and muscle (0.14 µg, 0.19 µg and 0.93 µg respectively).

8. 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
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)
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer

Problem	Reason	Solution
	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)
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

Problem	Reason	Solution
	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

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

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www.abcam.co.jp/contactus (Japan)