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ab272532
Glucose Assay Kit

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Glucose Assay Kit datasheet:
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For quantitative determination of Glucose concentration in biological samples.

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

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1. Overview

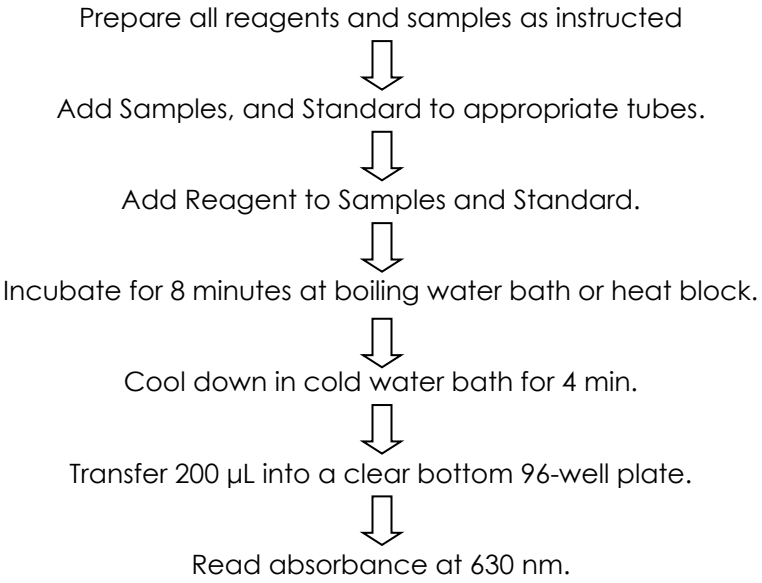
Glucose Assay Kit (ab272532) is a simple, direct and automation-ready procedure for measuring glucose concentrations find wide applications in research and drug discovery. This assay is designed to measure glucose directly in serum or plasma without any pretreatment. The improved o-toluidine method utilizes a specific color reaction with glucose. The absorbance at 630nm is directly proportional to glucose concentration in the sample.

Sensitive and accurate: Use as little as 5 µL samples. Linear detection range 0.7 mg/dL (39 µM) to 300 mg/dL (16.6 mM) glucose in 96-well plate.

Simple and convenient: The procedure involves addition of a single working reagent and incubation for 8 min in a boiling water bath. Improved reagent stability. The optimized formulation has greatly enhanced the reagent and signal stability.

Low interference in biological samples: No pretreatments are needed. Assays can be directly performed on serum and plasma samples.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store reagent at room temperature and standard at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 12 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Reagent	50 mL	Room temperature
Standard (300 mg/dL Glucose)	1 mL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Distilled H2O
- Multi-channel pipette
- 1.5 mL tubes
- 1.5 mL centrifuge
- 96-well clear plate with flat bottom (alternatively, 1 mL cuvettes may be used)
- Standard microplate reader - capable of reading absorbance at 620-650 nm (peak absorbance is at 630 nm).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate reagent to room temperature (18-25°C) prior to use.
- Reagent comes as is ready to use.
- The kit contains enough reagents for 100 assays.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare diluted standards immediately prior to use.

Standard Dilution:

10.1.1 Dilute standard in 1.5 mL centrifuge tubes as described in the table, below.

Standard #	Standard (µL)	dH_2O (µL)	Glucose Conc. (mg/dL)
1	150	0	300
2	100	50	200
3	50	100	100
4	25	125	50
5	0	150	0

- Store diluted standards at -20°C for future use.

11.Sample Preparation

Sample treatment:

11.1.1 Samples can be analyzed immediately after collection.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

Procedure using 96-well plate:

12.1.1 Set up 1.5-mL centrifuge tubes.
12.1.2 Add 5 µL of Diluted Standards (#1,2,3,4,5,) to labeled tubes.
12.1.3 Add 5 µL of samples to labelled tubes
12.1.4 Add 500 µL of Reagent to each tube and close tubes tightly and mix.

ΔNote: The reagent contains acetic acid. This assay is preferably carried out in a chemical fume hood.

12.1.5 Place the tubes in a tube holder and heat in a boiling water bath or heat block for 8 min.
12.1.6 Cool down in cold water bath for 4 min.
12.1.7 Transfer 200 µL into a clear bottom 96-well plate.

ΔNote: Avoid bubble formation.

12.1.8 Read OD at 620-650 nm (peak 630 nm).

Procedure using cuvette:

12.1.9 Add 12 µL of H_20 (Blank), Diluted Standards and samples to appropriately labeled tubes.
12.1.10 Add 1200 µL of Reagent to each tube. Close the tubes tightly and mix.
12.1.11 Place the tubes in a tube holder and heat in a boiling water bath for 8 min.
12.1.12 Cool down in cold water bath for 4 min
12.1.13 Transfer 1000 µL reaction mixture into cuvet.
12.1.14 Read OD at 620-650 nm (peak 630 nm) against blank.

ΔNote: If the Sample OD is higher than the Standard OD at 300mg/dL, dilute sample in water and repeat the assay.

ΔNote: To determine low glucose concentrations, use 50 µL sample and standards (instead of 5 µL) per 500 µL Reagent.

13. Calculations

13.1.1 Subtract Blank OD (#5) from the Standard and Sample OD values.
13.1.2 Plot the OD against standard concentrations.
13.1.3 Determine the slope using linear regression fitting.
13.1.4 The total Glucose concentration of Sample is calculated as

$$\frac{OD_{SAMPLE} - OD_{BLANK}}{Slope} \ (mg/dL)$$

OD_{SAMPLE} is OD values of sample.

OD_{BLANK} is OD values of H_2O.

Typical serum/plasma glucose values: 70 - 110 mg/dL.

Δ Note: 1mg/dL glucose equals 55.5 µM, 0.001% or 10 ppm.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.

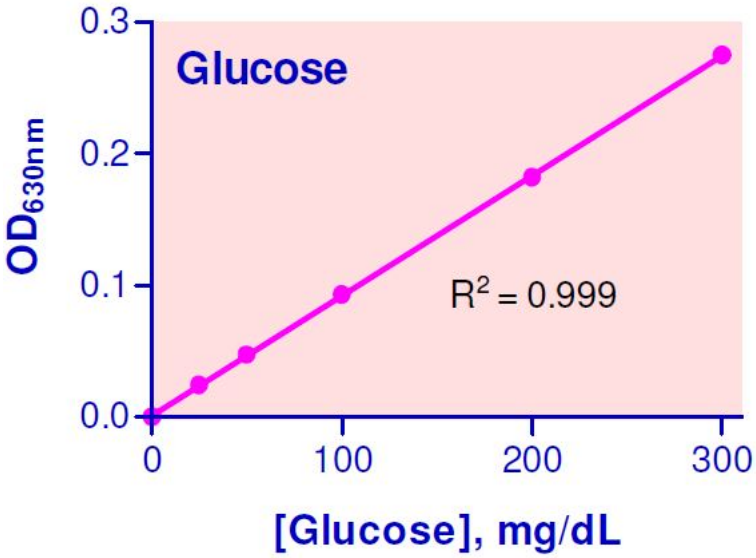


Figure 1. Example of Glucose Assay Kit standard curve.

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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