Instructions for Use

Version: 1.0.1
Revision date: 16-Jul-24

abbexa®

Glucose (Glu) Assay Kit

Catalogue No: abx298860

Size: 96 tests

Storage: Store Phenol Solution and Enzyme solution in the dark at 4°C and the rest of the components at 4°C.

Application: For detection and quantification of Glucose (Glu) in whole blood, serum, plasma, tissue samples

Introduction

Abbexa's Glucose Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Glucose content. Glucose oxidase can catalyse the oxidation of glucose to gluconic acid to produce hydrogen peroxide. In the presence of chromogenic oxygen receptors, peroxidase catalyses hydrogen peroxide and oxidises pigment sources to form coloured substances. The product has an absorbance maximum at 505 nm. The intensity of the colour is proportional to the Glucose concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Phenol solution 20 ml
3. Enzyme solution: 20 ml
4. Glucose standard (50 mmol/L): 1.2 ml
5. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (505 nm)
2. Double distilled water
3. Normal saline (0.9 % NaCl)
4. Pipette and sterile pipette tips
5. Centrifuge
6. Vortex mixer
7. Incubator

For Reference Only

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyse immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and plasma:** Samples can be detected directly after collection, separation and treatment. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue homogenates:** Weigh > 20 mg of tissue and wash with pre-chilled normal saline (0.9 % NaCl). For each 1 mg of tissue, add 9 µl of normal saline (0.9% NaCl) and homogenize using a mechanical homogenizer, or by ultrasonication at 4°C (for example, add 180 µl of Normal saline to 20 mg of tissue). Centrifuge the homogenate at 10,000 x g at 4°C for 10 min, then collect the supernatant and store on ice for immediate detection. The protein concentration in the supernatant should be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl).

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human Serum	1
Mouse Serum	1
Rat Serum	1
Human plasma	1

Preparation of the standard curve:

Label 7 tubes with 2 mmol/l, 5 mmol/l, 10 mmol/l, 15 mmol/l, 20 mmol/l, 25 mmol/l, and 30 mmol/l. Add 60 µl, 50 µl, 40 µl, 30 µl, 20 µl, 10 µl, and 4 µl of Standard (50 mmol/l) to the 30 mmol/l, 25 mmol/l, 20 mmol/l, 15 mmol/l, 10 mmol/l, 5 mmol/l, and 2 mmol/l tubes respectively, followed by 40 µl, 50 µl, 60 µl, 70 µl, 80 µl, 90 µl, and 96 µl of Distilled water, to prepare the Standard Solutions with concentrations 30 mmol/l, 25 mmol/l, 20 mmol/l, 15 mmol/l, 10 mmol/l, 5 mmol/l, and 2 mmol/l. These volumes are summarized in the following table:

Concentration (mmol/L)	**2**	**5**	**10**	**15**	**20**	**25**	**30**
Volume of 50 mmol/L glucose standard (µl)	**4**	**10**	**20**	**30**	**40**	**50**	**60**
Double distilled water (µl)	**96**	**90**	**80**	**70**	**60**	**50**	**40**

For the blank, or 0 mmol/L standard, use pure double distilled water. The volume of each standard will be 100 µl.

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Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

Assay Procedure

1. Set standard, blank, sample and control wells on the microplate and record their positions. Controls are required for whole blood and haemolysis samples only. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
2. Add 3 µl of standard solution to the standard wells.
3. Add 3 µl of sample to the sample wells
4. Add 3 µl of sample to the control wells
5. Add 300 µl of enzyme working solution to the standard and sample wells.
6. Add 300 µl of control working solution to the control wells.
7. Cover the plate with a plate sealer. Gently tap the plate to mix, or use a microplate shaker. Incubate at 37°C for 15 minutes.
8. Measure the OD of each well with a microplate reader at 505 nm.

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Calculation of Results

1. Average the duplicate readings for each standard.
2. Subtract the mean OD value of the blank from all standard readings. This is the absolute OD value.
3. Plot the standard curve, using the average OD of the standard dilutions on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Glucose in each sample well can be derived with the formulas below.

1. Normal serum/plasma samples:

$$\text{Glucose (mmol/L)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) - b}{a} \times f$$

2. Whole blood and haemolysis samples:

$$\text{Glucose (mmol/L)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - b}{a} \times f$$

3. Tissues samples:

$$\text{Glucose (mmol/g)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) - b}{a\ x\ C} \times f$$

where:

a	gradient of the standard curve $y = ax + b$
b	intercept of the standard curve $y = ax + b$
$\text{OD}_{\text{Sample}}$	OD value of sample
$\text{OD}_{\text{Control}}$	OD value of control
OD_{Blank}	OD value of blank
C	Concentration of protein in sample (g/L)
f	The dilution factor of sample

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D. Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.