Glucose Assay Kit

Catalog No.: abx097991

Size: 96 tests

Detection Range: 0.1 µmol/L – 20 µmol/L

Sensitivity: 0.1 µmol/L

Storage: Store all components at -20°C. Store the Chromogenic Reagent and Enzyme Reagent in the dark.

Application: For detection and quantification of Glucose concentration in serum, plasma, urine, saliva, milk, and cell lysates.

Principle of the Assay

Abbexa's Glucose Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Glucose concentration. Glucose is oxidized by glucose oxidase to produce gluconic acid and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with a probe to generate a fluorescent product with an excitation wavelength of 535 nm and an emission wavelength of 590 nm. The intensity of the fluorescence is proportional to the glucose concentration, which can then be calculated.

Kit components

1. Black 96-well microplate
2. Buffer Solution: 2 × 50 ml
3. Enzyme Reagent: 1 vial
4. Chromogenic Reagent: 0.25 ml
5. Standard (5 mmol/L): 0.5 ml
6. Plate sealer: 2

Materials required but not provided

1. Fluorescence Microplate reader (Ex 535 nm/Em 590nm)
2. PBS (0.01M, pH 7.4)
3. Pipette and pipette tips
4. Centrifuge and centrifuge tubes
5. Sonicator
6. Vortex mixer
7. Incubator

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Protocol

A. Preparation of samples and reagents

1. Reagents

- **Emzyme Working Solution:** Reconstitute 1 vial of Emzyme Reagent with 250 µl of Buffer Solution.Mix thoroughly until fully dissolved. Unused Enzyme Working Solution can be stores at -20°C for up to 1 month in the dark.
- **Chromogenic Working Solution:** Prepare enough Chromogenic Working Solution for the wells to be tested. Mix Chromogenic Reagent, Enzyme Working Solution and Buffer Solution in a ratio of 1 : 1 : 23. For example, prepare 50 µl of Chromogenic Working Solution by mixing 2 µl of Chromogenic Reagent, 2 µl of Enzyme Wokring Solution and 46 µl of Buffer Solution. The Chromogenic Working Solution should be prepared immediately before use and protected from light.
- **50 µmol/L Standard:** Dilute the stock Standard (5 mmol/L)100-fold with Buffer Solution. For example, add 7 µl of Standard (5 mmol/L) to 693 µl of Buffer Solution and mix thoroughly. Unused 50 µmol/L Standard can be stored at 4°C for up to 7 days.
- **Standards:** Label 7 tubes with 8 µmol/L, 7 µmol/L, 6 µmol/L, 5 µmol/L, 4 µmol/L, 2 µmol/L, and 1 µmol/L. Prepare these dilutions according to the volumes in the following table:

Standard Dilution (µmol/L)	**8**	**7**	**6**	**5**	**4**	**2**	**1**
50 µmol/L Standard (µl)	160	140	120	100	80	40	20
Buffer Solution (µl)	40	60	80	100	120	160	180

For the blank, use Buffer Solution. The volume of each standard dilution will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Avoid repeat freeze-thawing of the Enzyme Wokring Solution by aliquoting prior to storage.

2. Samples

Isolate the test samples soon after collecting and analyze 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**, **plasma and other liquid fluids:** If the sample is cloudy,, centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully collect the supernatant and keep it on ice for detection. Unused sample can be stored at -80°C for up to 1 month.

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- **Cell samples:** collect at lease 2 × 10^6 cells for analysis. Wash the cells with cold PBS (0.01M, pH 7.4). Per 2 × 10^6, add 200 µl of Buffer Solution. Sonicate at 4 °C for homogenization. Centifuge at 10,000 × g for 10 minutesat 4°C . Carefully collect the supernatant and keep on ice for detection.

  Note: To calculate Glucose concentration in cell samples using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (**abx097193**).

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 Buffer Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human plasma	300 – 600
Human serum	300 – 600
Chicken serum	600 – 1000
Human urine	1
Human milk	400 – 600
Saliva	3 – 5

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

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B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Mark positions on the 96-well microplate for each standard, blank and sample. *It is strongly recommended to prepare all wells in duplicate.*
2. Add 50 µl of sample to each sample well.
3. Add 50 µl of Buffer Solution to each blank well.
4. Add 50 µl of each standard dilution to the corresponding standard wells.
5. Add 50 µl of Chromogenic Working Solution to all wells.
6. Mix the well contents by tapping the plate, or shaking with a microplate shaker, for approximately 10 seconds.
7. Cover with a plate sealer, then incubate the plate at 37°C for 15 minutes in the dark.
8. Unseal the plate, then measure the fluorescence of each well with a fluorescence microplate reader with an excitation wavelength of 535 nm and an emission wavelength of 590 nm.

C. Calculation of Results

Plot the standard curve, using the fluorescence of the standard dilutions (adjusted for the blank) 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 following formulae:

1. Serum, Plasma and othe liquid fluids:

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

2. Cell samples:

$$\text{Glucose (µmol/g protein)} = \frac{(\text{F}_{\text{Sample}} - \text{F}_{\text{Blank}} - \text{b})}{\text{a}} \times \frac{\text{f}}{\text{C}_{\text{Protein}}}$$

where:

F_{Sample}	Fluorescence value of sample
F_{Blank}	Fluorescence value of blank
$\text{C}_{\text{Protein}}$	Concentration of protein in sample (g/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
f	The dilution factor of sample

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Technical Support

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