Glucose-6-phosphate (G6P) Assay Kit

Catalog No.: abx295038

Size: 96 tests

Detection Range: 5.6 µmol/L – 500 µmol/L

Sensitivity: 5.6 µmol/L

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

Application: For detection and quantification of Glucose-6-phosphate concentration in serum, plasma, and tissue homogenates.

Introduction

Abbexa's Glucose-6-phosphate Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Glucose-6-phosphate concentration. In the presence of glucose-6-phosphate dehydrogenase, glucose-6-phosphoric acid is oxidized to form gluconolactone-6-phosphate, whilst NADP+ is reduced to form NADPH. Further reduction as a result of the action of 1-MPMS, results in the formation of an orange compound, with an absorbance be calculated.ł50 nm. The intensity of the color is proportional to the Glucose-6-phosphate concentration, which can then

Kit components

1. 96-well microplate
2. Extraction Solution: 2 × 60 ml
3. Buffer Solution: 5 ml
4. Enzyme Reagent: 1 vial
5. Chromogenic Reagent: 2 × 1.5 ml
6. Standard (10 mmol/L): 0.5 ml
7. Plate sealer: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Centrifuge and centrifuge tubes
6. Vortex mixer
7. Incubator

Protocol

A. Preparation of samples and reagents

1. 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** and **Plasma:** Serum and plasma samples can be tested directly.
- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue, and wash in cold PBS (0.01 M, pH 7.4). For each 20 mg of tissue, add into 180 µl of Extraction Solution. Homogenize manually, using a mechanical homogenizer at 4°C. Collect the tissue homogenate, and centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully collect the supernatant and keep on ice for immediate detection.

  Note: To calculate Glucose-6-phosphate concentration in tissue homogenates 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 Extraction 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	1
Mouse serum	2 – 3
Rat plasma	1
Porcine serum	1
10% Rat spleen tissue homogenate	2 – 3
10% Rat heart tissue homogenate	2 – 3
10% Rat liver tissue homogenate	2 – 3
10% Mouse lung tissue	1

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.

2. Reagents

- **1 mmol/L Standard:** Dilute 38 µl of 10 mmol/L Standard with 342 µl of Extraction Solution and mix thoroughly. Prepare for immediate use.
- **Enzyme Working Solution:** Dissolve 1 vial of Enzyme reagent in 1.8 ml of Buffer Solution and mix thoroughly. Prepare for immediate use or store at -20°C for up to 7 days.
- **Control Working Solution**: Prepare only as much Control Working Solution as required for each control well tested. Add of Buffer Solution to of Chromogenic Reagent at a ratio of 1:1. For example to prepare 50 µl of Control Working Solution, add 25 µl of Buffer Solution to 25 µl of Chromogenic Reagent and mix thoroughly. Prepare for immediate use and keep in the dark.
- **Sample Working Solution** Prepare only as much Sample Working Solution as required for the wells tested. Add Chromogenic Reagent and Enzyme Working Solution in a ratio of 1:1. For example, to prepare 50 µl of Sample Working Solution, add 25 µl of Chromogenic Reagent to 25 µl of Enzyme Working Solution and mix thoroughly. Prepare for immediate use and keep in the dark.
- **Standards:** Label 7 tubes with 500 µmol/L, 400 µmol/L, 350 µmol/L, 300 µmol/L, 200 µmol/L, 100 µmol/L, and 50 µmol/L. Prepare the concentrations as summarized in the following table:

Standard Dilution (µmol/L)	**500**	**400**	**350**	**300**	**200**	**100**	**50**
1 mmol/L Standard (µl)	100	80	70	60	40	20	10
Extraction Solution (µl)	100	120	130	140	160	180	190

For the blank use pure Extraction Solution. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

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

1. Record the well locations for each standard, sample, blank and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the wells in duplicate.*
2. Add 50 µl of sample to each sample well, and 50 µl of the same sample to the corresponding control well.
3. Add 50 µl of each to standard dilution to the corresponding standard wells.
4. Add 50 µl of Sample Working Solution to the sample and standard wells.
5. Add 50 µl of Control Working Solution to the control wells.
6. Gently tap the plate or shake with a microplate shaker for 5 seconds to mix thoroughly.
7. Incubate at 37°C for 10 minutes.
8. Measure and record the OD of each well with a microplate reader at 540 nm.

C. Calculation of Results

Plot the standard curve, using the OD 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-6-phosphate in each sample well can be derived with the following formulae:

1. Serum and Plasma samples:

$$\text{Glucose-6-phosphate Concentration } (\mu\text{mol/L}) = \frac{(\Delta A - b)}{a} \times F$$

2. Tissue homogenates:

Glucose-6-phosphate concentration in tissue samples can be calculated according to total protein concentration (which must be assayed separately).

Total Protein

$$\text{Glucose-6-phosphate concentration } (\mu\text{mol/g protein}) = \frac{(\Delta A - b)}{a \times C_{Protein}} \times F$$

where:

ΔA	$A_1 - A_2$
A_1	OD value of sample
A_2	OD value of control
$C_{Protein}$	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

Technical Support

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