Glucose-6-Phosphate Dehydrogenase (G6PD) Assay Kit

Catalog No.: abx298812

Size: 96 tests

Detection Range: 0.01 U/L – 50 U/L

Sensitivity: 0.01 U/L

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

Application: For detection and quantification of Glucose-6-Phosphate Dehydrogenase activity in serum, plasma, saliva, and tissue homogenates.

Introduction

Abbexa's Glucose-6-Phosphate Dehydrogenase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Glucose-6-Phosphate Dehydrogenase activity. Glucose-6-Phosphate Dehydrogenase in samples oxidizes Glucose-6-Phosphoric Acid, while NADP+ is reduced to NADPH. NADPH reduces WST-8 to produce an orange-colored product with an absorbance maximum at 450 nm. The intensity of the color is proportional to the Glucose-6-Phosphate Dehydrogenase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Extraction Solution: 2 × 50 ml
3. Substrate: 2 × 1.5 ml
4. Chromogenic Reagent: 2 × 1.5 ml
5. Buffer Solution: 4 ml
6. Standard: 1 vial
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. 1.5 ml microcentrifuge tubes
6. Dounce homogenizer
7. Centrifuge
8. Vortex mixer
9. Water bath
10. 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/Plasma:** Samples can be tested directly. Samples can be stored at -80°C for up to 1 month.
- **Saliva:** Gargle with clear water, then collect saliva after 30 minutes. Centrifuge at 10,000 × g at 4°C for 10 minutes. Take the supernatant and store on ice for immediate assay.
- **Tissue Homogenates:** Carefully weigh 20 mg of tissue and wash with cold PBS (0.01 M, pH 7.4). Homogenize in 180 µl Extraction Solution using a Dounce homogenizer at 4°C, then centrifuge at 10,000 × g at 4°C for 10 minutes. Collect the supernatant and store on ice for immediate assay. The protein content of the supernatant should 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 serum	1
Rat serum	1
Rabbit serum	1
Cynomolgus monkey serum	1
10 % Mouse liver tissue homogenate	3-5
10 % Rat kidney tissue homogenate	1
10 % Rat spleen tissue homogenate	5-10
10 % Mouse brain tissue homogenate	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

- **Sample Working Solution:** For each sample, mix 25 µl of Substrate with 25 µl of Chromogenic Reagent. Prepare fresh before use.
- **Control Working Solution:** For each control sample, mix 25 µl of Buffer Solution with 25 µl of Chromogenic Reagent. Prepare fresh before use.
- **5 mmol/L Standard Solution**: Dissolve a vial of standard with 720 µl double distilled water and mix thoroughly. Prepare fresh before use.
- **500 µmol/L Standard Working Solution:** Dilute 5 mmol/L Standard Solution 10-fold with Buffer Solution. Prepare sufficient working solution for standard dilutions as described below.
- **Standard Dilutions:** Prepare Standard Working Solution dilutions as summarized in the following table:

Standard Dilution (µmol/L)	**0**	**50**	**100**	**150**	**250**	**350**	**400**	**500**
500 µmol/L Standard Working Solution (µl)	0	20	40	60	100	140	160	200
Extraction Solution (µl)	200	180	160	140	100	60	40	0

For the blank, or 0 µmol/L standard, use pure Extraction Solution. The volume of each standard will be 200 µl.

Note:

- Store Substrate on ice during use, allow all other reagents to equilibrate to room temperature before use.
- Avoid foaming when transferring solutions to microplate wells

B. Assay Procedure

1. Assign microplate wells for each standard, sample, and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 50 µl of standard dilutions to standard wells.
3. Add 50 µl of sample to sample wells.
4. Add 50 µl of sample to corresponding control wells.
5. Add 50 µl of Sample Working Solution to sample and standard wells.
6. Add 50 µl of Control Working Solution to control wells.
7. Mix thoroughly then incubate at 37°C for 10 minutes.
8. Measure the OD of each well with a microplate reader at 450 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 Dehydrogenase in each sample well can be derived with the following formulae:

1. Serum/Plasma/Saliva samples:

One unit of Glucose-6-Phosphate Dehydrogenase activity is defined as the amount of enzyme in 1 L of sample which produces 1 µmol of NADPH per minute at 37°C.

$$\textbf{Glucose-6-Phosphate Dehydrogenase Activity (U/L)} = \frac{\Delta \boldsymbol{A} - \boldsymbol{b}}{\boldsymbol{a} \times \boldsymbol{T}} \times \boldsymbol{f}$$

2. Tissue samples:

One unit of Glucose-6-Phosphate Dehydrogenase activity is defined as the amount of enzyme in 1 g of tissue protein which produces 1 µmol of NADPH per minute at 37°C.

$$\textbf{Glucose-6-Phosphate Dehydrogenase Activity (U/g protein)} = \frac{\Delta \boldsymbol{A} - \boldsymbol{b}}{\boldsymbol{a} \times \boldsymbol{T}} \times \frac{\boldsymbol{f}}{\mathbf{C_{Protein}}}$$

where:

ΔA	$OD_{Sample} - OD_{Control}$
f	Dilution factor of sample before carrying out the assay
T	Reaction time (10 minutes)
$C_{Protein}$	Concentration of protein in sample (g protein/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)