(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Glucose-6-phosphate (G6P) Assay Kit (WST-8 Method)

Catalog No.: BC00033

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Glucose-6-phosphate (G6P) Assay Kit (WST-8 Method)
Detection Method	Colorimetric
Sample Type	Tissues and cells
Assay Type	Quantitative
Detection Instrument	Microplate reader (450-490 nm, optimal detection wavelength 450 nm)
Range	20-500μM
Sensitivity	1.2607μM

Product Introduction

G6P is a molecule generated by phosphorylation of the hydroxyl group on the 6th carbon of glucose under the catalysis of hexokinase. It is a common small molecule in sugar metabolism in cells and participates in biochemical pathways such as glycolysis (glycolytic pathway) and pentose phosphate pathway (PPP). In the first step of glycolysis, glucose is catalyzed by hexokinase to produce glucose-6-phosphate, and then catalyzed by phosphoglucose isomerase to form fructose-6-phosphate to continue the other steps of glycolysis; in the pentose phosphate pathway, glucose-6-phosphate is its first substrate, and this process is also the main pathway for generating NADPH. In addition to these two metabolic pathways, glucose-6-phosphate can also be converted into glycogen or starch and stored.

Product Features

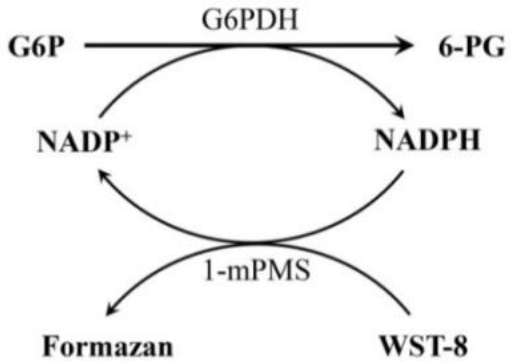
- WST-8 is an upgraded substitute for MTT, and has obvious advantages over MTT or other MTT-like products such as XTT, MTS, etc. First, the formazan generated by the reduction of MTT by some dehydrogenases is not water-soluble and requires a specific dissolving solution to dissolve; while the formazan generated by WST-8, XTT, and MTS are all water-soluble, which can save the subsequent dissolution step. Secondly, the formazan generated by WST-8 is more soluble than the formazan generated by XTT and MTS. Thirdly, WST-8 is more stable than XTT and

MTS, making the experimental results more stable.

- Compared with MTT, XTT, etc., WST-8 has a wider linear range and higher sensitivity.
- Compared with WST-1, WST-8 has higher detection sensitivity, is more soluble, and is more stable.
- This kit is easy to use, highly sensitive, and has a wide linear range. It can detect G6P as low as 20μM (1nmol), and shows a good linear relationship between 20μM (1nmol) and 500μM (25nmol). The detection can be performed using lysate of cells, tissues, etc., without the need to separate and purify G6P in cells, tissues, or other samples.

Principle

G6P is oxidized to generate 6-PG under the action of G6PDH. In this reaction process, $NADP^+$ is reduced to NADPH. The generated NADPH reduces WST-8 to generate orange-yellow formazan under the action of the electron coupling reagent 1-mPMS (1-Methoxy-5-methylphenaziniumMethylSulfate), with a maximum absorption peak at around 450nm. The formazan generated in the reaction system is directly proportional to the activity of G6PDH in the sample. The principle of WST-8 method for detecting G6P is shown in the figure below:



Components

No.	Components	Size (100T)	Storage

Reagent 1	Enzyme Mixture	220μl	-20℃
Reagent 2	G6P Standard (10mM)	100μl	-20℃
Reagent 3	Color Development Solution	220μl	-20℃, protect from light.
Reagent 4	Reaction Buffer	5.5ml	-20℃
Reagent 5	G6P Extract	50ml	-20℃
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20°C for 12 months.

Preparation

- **Sample handling**

1. Thaw the G6P extract at room temperature or in a 37ºC water bath, and place in an ice bath after thawing. If thawing in a 37ºC water bath, be sure to place in an ice bath immediately after thawing.
2. Preparation of cell samples: For adherent cells, about 1×10^6 cells (equivalent to the number of cells that grow in one well of a 6-well plate), aspirate the culture medium, add 200μl of ice-cold G6P extract with a pipette, and gently blow to promote cell lysis; for suspended cells, collect about 1×10^6 cells, centrifuge at 600g for 5 minutes, aspirate the culture medium, add 200μl ice-cold G6P extract with a pipette, and gently blow to promote cell lysis. Then centrifuge at 12,000g, 4ºC for 5-10 minutes, take the supernatant as the sample to be tested, and store it in an ice bath for later use.
3. Preparation of tissue samples: After washing the tissue with ice-cold PBS, weigh about 10-30 mg of tissue sample, chop it with scissors, put it in a homogenizer, add 400 μl of

ice-cold G6P extract solution and homogenize it on ice or at room temperature. Then centrifuge at 12,000g, 4ºC for 5-10 minutes, take the supernatant as the sample to be tested, and store it in an ice bath for later use. Note: The homogenization process can be performed on ice or at room temperature, but it is better to operate it in an ice bath, which can effectively reduce the decrease in endogenous G6P levels.

- **Preparation of the kit**

1. Preparation of G6P standard solution: Dilute 10mM G6P standard with G6P extract to an appropriate concentration gradient. For example, for the initial test, you can set the concentrations to 0, 31.25, 62.5, 125, 250, and 500μM. When testing, add 50μl of standard to each well of the 96-well plate, which is equivalent to adding 0, 1.56, 3.125, 6.25, 12.5, and 25nmol of G6P to each well. If necessary, in subsequent experiments, the concentration range of the standard can be appropriately adjusted according to the G6P content in the sample. The point with a concentration of 0μM is the blank control point (Blank), which only contains G6P extract.
2. Preparation of G6P detection solution: NADPH and other substances in the sample may produce a certain background. It is recommended to set up a background control by adding the sample but not the enzyme mixture. For the G6P detection solution of the standard and sample, the enzyme mixture needs to be added. Each background control, standard or sample requires 50μl of G6P detection solution. Please prepare an appropriate amount of G6P detection solution according to the number of background controls, standards and samples to be tested, and make sure to prepare it before use. The preparation method of G6P detection solution is as follows (the color developing solution must be properly mixed before use):

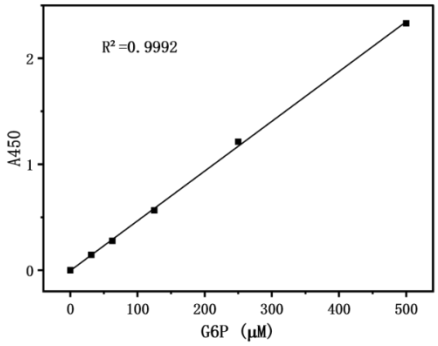
	G6P detection solution (background control)	G6P detection solution (standard or sample)
Reaction buffer	48μl	46μl
Color development solution	2μl	2μl
Enzyme mixture	- -	2μl

Operation process

1. Determination of G6P content in samples: Pipette 50μl of the sample to be tested into a 96-well plate. In order to reduce experimental errors, it is recommended to set up duplicate wells for the sample. If the G6P content in the sample is found to be too high and exceeds the range of the standard curve of the standard, the sample needs to be appropriately diluted with G6P extract before testing; if the content is too low, the amount of cell or tissue sample needs to be increased.
2. Add 50μl of G6P detection solution to each well and gently blow it evenly with a pipette. Be gentle when adding G6P detection solution to avoid bubbles. If bubbles appear accidentally, use a small pipette tip or needle to puncture them. 50μl of G6P detection working solution without G6P substrate needs to be added to the background control well. Special attention: If the background generated by NADPH in the sample is relatively high, a background control must be set; a background control should be set for the initial detection.
3. Incubate at 37 º C in the dark for 10 minutes. Orange-yellow formazan will be formed. Measure the absorbance at 450nm (450nm-490nm is acceptable, 450nm is optimal). If the color is light, the incubation time can be extended to 15-30 minutes. The color will become darker as the incubation time is extended.

Calculation

1. Subtract the absorbance of the blank control (Blank) with a standard concentration of 0 μM from the absorbance of the standard and sample. At the same time, if the absorbance of the background control (Background) is relatively high, it is necessary to subtract the absorbance of each background control from the absorbance of all samples.
2. The G6P concentration is the horizontal axis and the absorbance is the vertical axis to draw a standard curve. Please refer to the following figure for the detection effect of the G6P standard:



The reaction time in the figure is 30 minutes. Under different test conditions, the actual readings will vary due to the preparation of the standard, the detection instrument, etc. The data in the figure is for reference only.

3. Calculate the G6P concentration in cells, tissues and other samples based on the standard curve. Note: The amount of G6P can be calculated based on the concentration obtained and the volume of the sample.
4. If you want to express the amount of G6P more accurately, you can use the protein concentration determination kit (BCA method) (BC00006) produced by EnkiLife to measure the protein concentration of the sample. Finally, the amount of G6P per unit protein is used to express it more accurately.

Notes

1. The enzyme activity of the enzyme mixture in this kit was not affected by storage at room temperature for 72 hours or repeated freezing and thawing for 5 times.
2. Since the G6P extract is slightly viscous, when using this extract as a diluent, whether diluting the standard or the sample, be sure to ensure that the dilution is uniform during the dilution process, otherwise it will easily cause large fluctuations in the experimental data.
3. During sample addition and mixing, try to avoid generating bubbles to avoid affecting the final absorbance measurement.
4. If the reaction temperature and reaction time cannot be strictly controlled, a standard curve needs to be set up for each test.
5. If the G6P concentration in the sample solution is too high or too low and is not within the linear detection range of the kit, the amount of sample or extract can be appropriately adjusted.

6. Try to use fresh samples for testing. Frozen or repeatedly frozen-thawed cell or tissue samples may have a certain impact on the results.
7. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.