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

Glucose-6-Phosphate Dehydrogenase (G6PDH) Assay Kit (WST-8 Method)

Catalog No.: BC00032

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 Dehydrogenase (G6PDH) Assay Kit (WST-8
Detection Method	Colorimetric
Sample Type	Tissues and cells
Assay Type	Enzyme activity
Detection Instrument	Microplate reader (450-490 nm, optimal detection wavelength 450 nm)
Range	0-500mU/mL
Sensitivity	0.7962mU/mL

Product Introduction

G6PDH catalyzes the conversion of glucose-6-phosphate (G6P) into 6-phosphogluconolactone. ate, 6-PG), which is the first step of the pentose phosphate pathway (PPP) and the rate-limiting step of the pathway. The pentose phosphate pathway is essential for the production of NADPH (reduced nicotinamide adenine dinucleotide phosphate, also known as reduced coenzyme II) and pentoses. NADPH is essential for regulating redox balance and fatty acid biosynthesis through the regeneration of GSH. Therefore, the deficiency of G6PDH can lead to some diseases caused by the inability to generate NADPH, such as neonatal jaundice, non-immune hemolytic anemia, etc.

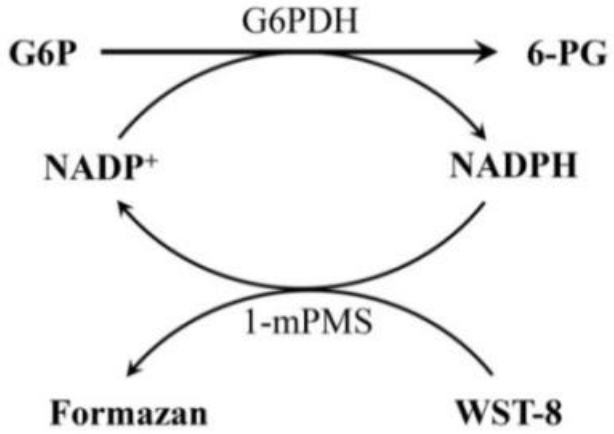
Product Features

- 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 G6PDH with a content as low as 0.05mU per well, and a good linear relationship between 1mU/ml (0.05mU/well) and 500mU/ml (25mU/well). The detection can be performed using lysate of cells, tissues, etc., without the need to separate and purify G6PDH 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 G6PDH is shown in the figure below:



Components

No.	Components	Size (100T)	Storage

Reagent 1	Reaction Buffer	5.5ml	-20℃
Reagent 2	G6PDH Substrate	220μl	-20℃
Reagent 3	Color Development Solution	220μl	-20℃, protect from light.
Reagent 4	G6PDH (0.25 U/μl)	25μl	-20℃
Reagent 5	G6PDH 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 G6PDH 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 (approximately equivalent to the number of cells that grow in one well of a 6-well plate), aspirate the culture medium, add 200μl of ice-bath pre-cooled G6PDH 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-bath pre-cooled G6PDH 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. Note: The lysis process can be performed on ice or at room temperature, but it is better to operate in an ice bath, which

can effectively reduce the decrease in enzyme activity caused by endogenous proteases.

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 G6PDH 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 enzyme activity caused by endogenous proteases.

- **Preparation of the kit**

1. Preparation of G6PDH standard: Mix 4 μl G6PDH (0.25 U/μl, i.e. 250 U/ml) with 996 μl G6PDH extract to make a 1U/ml G6PDH standard. Note: The diluted G6PDH is not very stable and should be used as soon as possible after preparation.
2. Setting up the G6PDH standard curve: Take 200μl of G6PDH standard (1U/ml) and use G6PDH extract to serially dilute it 3 times to form an appropriate concentration gradient. For example, for the initial test, you can set the concentrations of 0, 1.37, 4.1, 12.3, 37, 111, 333, and 1000mU/ml. When testing, add 50μl of different concentrations of standard to each well of the 96-well plate, which is equivalent to the amount of G6PDH enzyme added to each well of 0, 0.069, 0.21, 0.62, 1.85, 5.56, 16.7, and 50mU. If necessary, in subsequent experiments, the concentration range of the standard can be appropriately adjusted according to the G6PDH activity in the sample. The standard with a concentration of 0mU/ml is the blank control (Blank), which only contains G6PDH extract.
3. Preparation of G6PDH 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 samples but not G6PDH substrates. For G6PDH detection solutions of standards and samples, G6PDH substrates need to be added. Each background control, standard or sample requires 50μl of G6PDH detection solution. Please prepare an appropriate amount of G6PDH 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 G6PDH detection solution is as follows (the color developing solution must be properly mixed before use):

	G6PDH detection solution (background control)	G6PDH detection solution (standard or sample)
Reaction buffer	48μl	46μl
Color development solution	2μl	2μl
G6PDH substrate	- -	2μl

Operation process

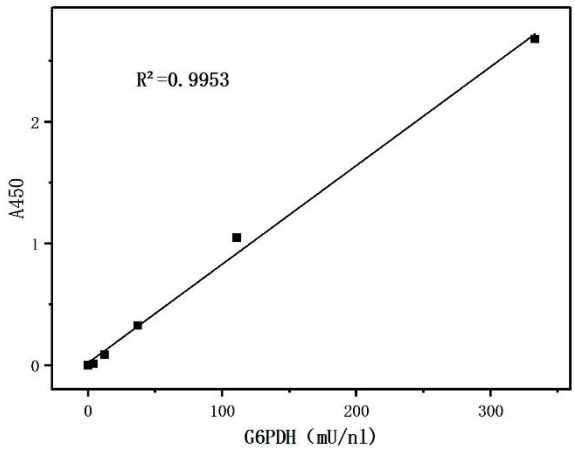
1. Determination of sample G6PDH activity: Pipette 50μl of the sample or standard to be tested into a 96-well plate. In order to reduce experimental errors, it is recommended to set up duplicate wells for the sample. If the G6PDH activity in the sample is found to be too high and exceeds the linear detection range of the standard, the sample needs to be appropriately diluted with G6PDH extract before testing; if the activity is too low, the amount of cell or tissue sample needs to be increased.
2. Add 50μl of G6PDH detection solution to each well of the sample or standard and mix appropriately. Add 50μl of G6PDH detection solution without G6PDH substrate to the background control well. Be gentle when adding G6PDH detection solution to avoid bubbles. If bubbles appear accidentally, use a small pipette or needle to puncture them. 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 first test.
3. Incubate at 37ºC 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 mU/ml 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 standard curve is drawn with G6PDH enzyme activity as the horizontal axis and absorbance as the vertical axis. Please refer to the figure below for the detection effect of G6PDH standard:



The concentrations of G6PDH in the figure are 0, 1.37, 4.1, 12.3, 37, 111, and 333 mU/ml, respectively, and the reaction time is 10 minutes. If the reaction time is appropriately shortened, a good linear relationship can be shown in the range of 0-500 mU/ml. Under different detection conditions, the actual readings will vary due to the preparation of the standard, the detection instrument, etc. The data in the figure are for reference only.

3. Calculate the G6PDH activity in cells, tissues and other samples according to the standard curve. Note: The activity unit of G6PDH can be calculated based on the activity obtained and the volume of the sample.
4. If you want to express the enzyme activity of G6PDH more accurately, you can use the protein concentration determination kit (BCA method) (BC00006) produced by EnkiLife to measure the protein concentration of cell or tissue samples prepared from G6PDH extract. Finally, the activity unit of G6PDH per unit protein amount is used to express it more accurately.

Notes

1. The G6PDH (from bacteria) in this kit has been tested to have no effect on its enzyme activity after being stored at room temperature for 72 hours or repeatedly frozen and thawed for 5 times. However, it is necessary to test whether the G6PDH of different

species can withstand long-term storage at room temperature or repeated freezing and thawing. Try to use freshly prepared samples for the first test.

2. Since the G6PDH extract is slightly viscous, when using the 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 absolute activity of G6PDH in a sample needs to be determined but the reaction temperature and reaction time cannot be strictly controlled, a standard curve needs to be set for each test.
5. If the G6PDH activity 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. 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.