WST-8 Glucose Uptake Assay Kit

Introduction

Glucose metabolism is a process that converts ingested glucose into energy, which is the main source of energy supply in living organisms. Studies have shown that glucose metabolism is closely related to many diseases such as cancer, diabetes and obesity, so it is important to monitor glucose uptake.

The WST-8 Glucose Uptake Assay Kit is a kit for detecting glucose uptake based on the WST-8 chromogenic reaction. The glucose analogue 2-DG can be uptaken into cells by glucose transporters and converted to 2-DG6P by endogenous enzymes. G6PDH catalyzes 2-DG6P into 6PGL, as well as the conversion of NADH+ to NADPH. NADPH can react with WST-8 to yield an orange-yellow formazan with a maximum absorption peak at 450 nm. Its absorbance is directly proportional to the ability of glucose uptake.

This kit can sensitively, rapidly and non-radioactively detect glucose uptake. The kit has a good linearity at concentrations of 10-500 μM.

Components and Storage

Components \ Size	100 Assays	500 Assays	Storage
Glucose Uptake Lysis Buffer	15 mL	70 mL	-20°C
Glucose Uptake Assay Buffer	10 mL	50 mL	-20°C
KRPH Buffer	12 mL	60 mL	-20°C
Glucose Uptake Reagent A	200 μL	1 mL	-20°C away from light
Glucose Uptake Reagent B	100 μL	500 μL	-20°C
Enzyme Solution	100 μL	500 μL	-20°C
2-DG (10 mM)	1 mL	5 mL	-20°C
2-DG6P Standard (10 mM)	50 μL	250 μL	-20°C
Shipping: Blue ice	Shelf life: 1 year		

Protocol

1. **Preparation before the experiment:**

   1) Warm Glucose Uptake Lysis Buffer, Glucose Uptake Assay Buffer, KRPH Buffer, 2-DG (10 mM) to room temperature in advance. Thaw Glucose Uptake Reagent A, Glucose Uptake Reagent B, and Enzyme

Solution and keep them on ice. Once the experiment is done, put the reagents back.

2) **Preparation of working solution:** Refer to the following table to prepare the working solution, the working solution needs to be stored on ice or 4°C in the dark and used within a few hours. Prepare a fresh working solution every time.

Number of samples	1	10	50
Glucose Uptake Assay Buffer	46 µL	460 µL	2300 µL
Glucose Uptake Reagent A	2 µL	20 µL	100 µL
Glucose Uptake Reagent B	1 µL	10 µL	50 µL
Enzyme Solution	1 µL	10 µL	50 µL
Total	50 µL	500 µL	2500 µL

***Note:** Because the latter three are used in smaller amounts and are easy to settle, they must be mixed well after addition.

2. Cell sample preparation

Here use oleic acid-induced Hela cells as an example, and the optimal protocol for other cells may need to be optimized.

1) Plate 10,000 cells per well in 96-well plates and culture for 24 h.

2) Remove the cell culture medium, add 100 µL of medium containing 800 µM oleic acid per well, and continue to culture for 24 h.

3) Wash twice with PBS, add 100 µL of serum-free low-glucose medium to each well, and incubate overnight.

***Note:**

a) Glucose-free medium can also be used, but treat cells with the glucose-free medium for no more than 6 h to avoid cell damage.

b) For differentiated 3T3-L1 adipocytes, only need serum-free starvation and starvation time can be optimized.

4) Next day, remove the medium, add 100 µL of KRPH Buffer. Then incubate at 37°C for 40 min.

5) For the background control group, do not treat cells with any drug or 2-DG. For the experimental group, treat cells with interested drug for desired time, then add 10 µL of 2-DG (10 mM) and incubate for 20 min.

***Note:** For some cells with slower 2-DG uptake, the incubation time of 2-DG can be increased appropriately, or the amount of 2-DG can be increased by adding 20 µL of 2-DG (10 mM).

6) (Optional) Set up a positive control treated with Insulin (e.g., 300 µg/mL) and a negative control treated with Phloretin (e.g., 150 µM) for 20 min. Then add 10 µL of 2-DG (10 mM) and incubate for 20 min.

***Note:** We also provide Insulin (B7407) and Phloretin (A3723).

7) Wash cells 3 times with PBS. Subsequently, add 100 µL of Glucose Uptake Lysis Buffer to each well and pipette to promote cell lysis. Centrifuge at 4°C,14000 g for 5 min, take the supernatant for assays. Perform all of the above steps on ice. Store the sample at -20°C or -80°C if it cannot be tested immediately.

3. Tissue sample preparation

1) Inject mice with an appropriate amount of 2-DG after treatment with the interested drug or genetic manipulation, and set mice without 2-DG injection as background control. In addition, it is recommended to set up positive and negative controls for experiments.

***Note:** It is recommended to inject an appropriate amount of glucose, which can reduce the interference of exogenous 2-DG on glucose metabolism in vivo.

2) According to the experimental design, take the tissue samples after a certain period (e.g., 30 min, 60 min, or 120 min) after 2-DG injection.

3) Homogenize 10 mg tissue sample per 100 μL of Glucose Uptake Lysis Buffer. Centrifuge at 4°C, 14000 g for 5 min, and take the supernatant for assay.

4. Preform the assay

1) Add 10 μL of 2-DG6P Standard (10 mM) to 190 μL of Glucose Uptake Assay Buffer, mix well to make 2-DG6P Standard (500 μM). Transfer 0, 0.5, 1, 2.5, 5, 10, 25, 50 μL of 2-DG6P Standard (500 μM) into 96-well plate, and then supplement with Glucose Uptake Assay Buffer to 50 μL. The standard concentrations are 0, 5, 10, 25, 50, 100, 250, 500 μM.

2) Add 1-50 μL of sample or diluted sample to the 96-well plate, and also make up the volume to 50 μL with Glucose Uptake Assay Buffer.

***Note:** To ensure that the sample value falls within the standard curve, it is recommended to make multiple dilutions of the sample, at which point the dilution factor can be denoted as n. If a 10-fold dilution is applied to the sample, 10 μL of the diluted sample is used, then n = 10×50/10 = 50.

3) Add 50 μL of working solution to each well, mix well, and incubate at 37°C for 30 min in the dark. The optimal incubation time can be optimized. If the absorbance is low, extend the incubation time appropriately.

4) Detect the absorbance (A) at 450 nm.

5) First establish the standard curve y=ax+b, and calculate the concentration of 2-DG6P (C).

$$C=(\Delta A-b)\div a\times n$$

Note: $\Delta A= A_{sample}- A_{background\ control}$; n is the dilution factor

Note

1. The dosage of 2-DG in this kit only meets cell experiments. For animal assays, it is recommended to purchase some more 2-Deoxy-D-glucose (Cat. No. B1027).

2. For your safety and health, please wear lab coats and gloves during the experiment.

3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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