

## Glucose Uptake Assay Kit (DTNB)

### 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.

This kit is designed for the detection of glucose uptake based on the DTNB chromogenic reaction. The glucose analog 2-deoxyglucose (2-DG) is taken up into cells via glucose transporters and is subsequently phosphorylated to 2-deoxyglucose-6-phosphate (2-DG6P) by endogenous kinases. Glucose-6-phosphate dehydrogenase (G6PDH) then catalyzes the conversion of 2-DG6P, while simultaneously reducing  $\text{NADP}^+$  to NADPH. In the presence of glutathione reductase (GR), NADPH reduces oxidized glutathione (GSSG) to reduced glutathione (GSH). The generated GSH then reacts with the chromogenic substrate DTNB to produce yellow-colored TNB, which has a maximum absorbance at 412 nm. The absorbance at 412 nm is directly proportional to the cellular glucose uptake capacity.

This kit enables sensitive, rapid, and non-radioactive measurement of glucose uptake. It has been validated to exhibit a good linear relationship within the range of 1 to 100 pmol. Compared with the WST-8 Glucose Uptake Assay Kit (Cat. No. K2303), this kit offers higher sensitivity and is therefore more suitable for samples with relatively low glucose uptake activity.

### Components and Storage

Size	100 Assays	500 Assays	Storage
<b>Components</b>			
Glucose Uptake Lysis Buffer	15 mL	70 mL	-20°C
Glucose Uptake Assay Buffer	10 mL	50 mL	-20°C
KRPB Buffer	12 mL	60 mL	-20°C
Enzyme Solution A	500 $\mu\text{L}$	2.5 mL	-20°C
Reaction Mix	200 $\mu\text{L}$	1 mL	-20°C
Stop Buffer	600 $\mu\text{L}$	3 mL	-20°C
GR Buffer	600 $\mu\text{L}$	3 mL	-20°C
Recycling Mix	200 $\mu\text{L}$	1 mL	-20°C
Enzyme Solution B (15X)	20 $\mu\text{L}$	100 $\mu\text{L}$	4°C
DTNB (10X)	220 $\mu\text{L}$	1.1 mL	-20°C away from light

2-DG (10 mM)	1 mL	5 mL	-20°C
2-DG6P Standard (1 mM)	20 µL	60 µL	-20°C
Shipping: Blue ice		Shelf life: 1 year	

## Protocol

### 1. Preparation before the experiment

- Warm Glucose Uptake Lysis Buffer, Glucose Uptake Assay Buffer, KRPB Buffer, Stop Buffer, GR Buffer and 2-DG (10 mM) to room temperature in advance. Thaw Reaction Mix, Recycling Mix, Enzyme Solution A, Enzyme Solution B (15X), DTNB (10X) and keep them on ice. Once the experiment is complete, return the reagents to their original locations.
- Preparation of working solution A: Refer to the table below to prepare working solution A, the working solution A needs to be stored on ice or 4°C in the dark and used within a few hours. Prepare a fresh working solution A every time.

Number of samples	1	10	50
Glucose Uptake Assay Buffer	14 µL	140 µL	700 µL
Enzyme Solution A	4 µL	40 µL	200 µL
Reaction Mix	2 µL	20 µL	100 µL
Total	20 µL	200 µL	1000 µL

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

- Preparation of working solution B: Refer to the table below to prepare working solution B, the working solution B needs to be stored on ice or 4°C in the dark and used within a few hours. Prepare a fresh working solution B every time. Dilute Enzyme Solution B (15X) with Glucose Uptake Assay Buffer to make Enzyme Solution B (1X) before use.

Number of samples	1	10	50
Glucose Uptake Assay Buffer	20 µL	200 µL	1000 µL
Enzyme Solution A	1 µL	10 µL	50 µL
Recycling Mix	2 µL	20 µL	100 µL
Enzyme Solution B (1X)	2 µL	20 µL	100 µL
Total	25 µL	250 µL	1250 µL

**\*Note:** Because the latter three are used in small quantities and are easy to settle, they must be mixed well after adding.

- DTNB working solution preparation: dilute an appropriate amount of DTNB (10X) in Glucose Uptake Assay Buffer at a ratio of 1:9 to obtain DTNB working solution.

### 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) Seed 10,000 cells per well in 96-well plates and culture for 24 h.
- 2) Remove the cell culture medium, add 100  $\mu$ L of medium containing 800  $\mu$ M oleic acid per well, and continue to culture for 24 h.
- 3) Wash twice with PBS, add 100  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L of 2-DG (10 mM).

- 6) (Optional) Set up a positive control treated with Insulin (e.g., 300  $\mu$ g/mL) and a negative control treated with Phloretin (e.g., 150  $\mu$ M) for 20 min. Then add 10  $\mu$ L 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  $\mu$ L of Glucose Uptake Lysis Buffer to each well and pipette to promote cell lysis. Centrifuge at 4°C, 14000 g for 5 min, and 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  $\mu$ L of Glucose Uptake Lysis Buffer. Centrifuge at 4°C, 14000 g for 5 min, and take the supernatant for assay.

### 4. Perform the assay

- 1) Add 1  $\mu$ L of 2-DG6P Standard (1 mM) to 249  $\mu$ L of Glucose Uptake Assay Buffer, mix well to make 2-DG6P Standard (4  $\mu$ M). Transfer 0, 1.25, 2.5, 5, 10, 15, 20, 25  $\mu$ L of 2-DG6P Standard (4  $\mu$ M) into 96-well plate to generate 0, 5, 10, 20, 40, 60, 80, 100 pmol/well of 2-DG6P. Then adjust the final volume

to 25  $\mu$ L/well with Glucose Uptake Assay Buffer.

- 2) Take 1-25  $\mu$ L of sample or diluted sample into a 96-well plate and also supplement it with the Glucose Uptake Assay Buffer to 25  $\mu$ L/well.

**\*Note:** To ensure that the sample readings fall within the standard curve, it is recommended to perform dilutions of the sample, at which point the dilution factor can be written as n. If a 10-fold dilution is applied to the sample, 10  $\mu$ L of the diluted sample is used, then  $n=10 \times 25 / 10 = 25$ .

- 3) Add 20  $\mu$ L working solution A to each well, mix well, and incubate at 37°C for 60 min.
- 4) Add 5  $\mu$ L Stop Buffer to each well, mix well, and incubate at 70°C for 60 min.
- 5) Add 5  $\mu$ L GR Buffer to each well, mix well
- 6) Add 25  $\mu$ L working solution B to each well and mix well.
- 7) Add 20  $\mu$ L DTNB working solution to each well and mix well.
- 8) Incubate at 37°C for 30-60 min. Measure  $A_{412}$  every 5 min until the reading of the highest concentration standard well (100 pmol) reaches 1.5-2.

**\*Note:** Under normal conditions, after 35 min of incubation, the signal of the highest concentration standard well (100 pmol) reaches approximately 1.5; after 45 min of incubation, the signal of the highest concentration standard well (100 pmol) reaches around 2.0.

- 9) Plot the standard curve  $y=ax+b$ , and calculate the amount of 2-DG (C).

$$C \text{ (pmol)} = (\Delta A - b) \div a \times n$$

Note:  $\Delta A = A_{\text{sample}} - A_{\text{background control}}$ ; n is the sample 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. Stop Buffer and GR Buffer are corrosive, so please pay attention to protection when using them.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.



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