Advanced BioReagents™

Glucose Uptake

For research use only

ABSbio™ Glucose Uptake Assay Kit (K958-100)

Quantitative Fluorescent Glucose Uptake Assay

Introduction

Evaluation of glucose uptake is crucial in the study of numerous diseases and metabolic disorders such as myocardial ischemia, diabetes mellitus, and cancer. Screening for glucose uptake inhibitor is a new way to search for cancer treatments. The ABSbio™ Glucose Uptake Assay Kit provides a simple, sensitive, fluorometric assay to evaluate the nonmetabolized glucose analog-2-deoxy-D-glucose (2DG) uptake in cultured cell. In this assay, 2-DG is taken up by glucose transporters and metabolized to 2-DG-6-phosphate (2-DG6P). The amount of the accumulated non-metabolizable 2-DG6P is directly proportional to glucose uptake by cells. The accumulated 2-DG6P is enzymatically coupled to generate NADPH, which is specifically monitored by a NADPH sensor at Ex/Em=530/590 nm. The kit is supplied with sufficient reagents for 100 tests in 96-well plate assay, linear detection range of 0.05-10 µM 2-DG6P use 50 µL sample volume. It could easily be modified for use in 384-well assay and high-throughput assay. It can be used for the high throughput screening of glucose uptake regulators and inhibitors.

Kit Components (100 tests)

Assay Buffer:	20 mL	Substrate:	0.12 mL	2-DG6P Standard:	0.1 mL	Enzyme Mix:	0.12 mL
Uptake Buffer:	20 mL	Probe:	0.15 mL	Neutralization Buffer:	6 mL	Extraction Buffer:	6 mL
2-DG (10 mM):	1 mL						

Storage and Handling: Shipping on icepack. Store kit at -20°C. Shelf Life: 12 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample preparation

1.1 **Prepare the cell culture**: Prepare differentiated cells, primary cell or stable cell lines expressing SGLT1/SGLT2 for 2-DG uptake assay in a 96-well culture plate with desired method.

1.2 **Stimulate the cell**: A) Maintain the cell in FBS-less/Glucose-free medium for 4-12 hr before adding the test agent. B) Remove the culture medium and replace with 100 µL test compound to the cell sample and incubate in a 5% CO_2 incubator at 37°C for a desired time. For control sample, do not treat cells with compound and 2-DG.

1.3 **2-DG uptake**: A) Wash the cell twice with PBS. B) Add 100 µL Uptake buffer containing 1 mM 2-DG, continue incubate the cells for 20-40 min. C) Wash the cells twice with PBS(after discarding the buffer, these cells can be stored in a freezer for up to 1 week before assay).

1.4 **Lyse the cells**: A) Add 50 µL Extraction buffer to each well and mix with a plate mixer. B) Heat the plate to 85°C in water bath for 40 min or transfer the lysate into 1.5 mL tube to heat on heat block. C) Neutralize the lysate by adding 50 µL Neutralization buffer.

1.5 Transfer 1-50 µL sample into a black 96-well clear flat bottom plate in duplicate and bring volume to 50 µL with Assay Buffer. For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Treat cells as desired(96-well plate)
↓
Culture cell
↓
Starving & treating cells
↓
Add 10 µL 10 mM 2-DG
↓
Add 50 µL Extraction buffer
↓
Heating degrade at 85°C
↓
Add 50 µL Neutralization buffer
↓
Add 150 µL Detection mixture
↓
Read Fluorescence

2. Standard Preparation

Dilute the 2-DG6P Standard to 10 µM by adding 2 µL of the 10 mM Standard to 1998 µL of dH_2O, mix well. Add 0, 5, 15, 30, 50 µL of the 10 µM Standard into a 96 well plate. Adjust volume to 50 µL/well with Assay Buffer to generate 0, 50, 150, 300, 500 pmole/well of 2-DG6P Standard. The blank control containing Assay Buffer only.

3. Reaction

Prepare enough reaction mixture by mixing 150 µL Assay Buffer, 1 µL Substrate, 1.5 µL Probe and 1 µL Enzyme Mix for each reaction (samples & standards). Transfer 150 µL prepared reaction mixture into each reaction well.

Tap plate to mix well. Incubate for 60 min. at 37°C, protected from light.

4. Measurement

Measure fluorescence at Ex/Em = 530/590 nm.

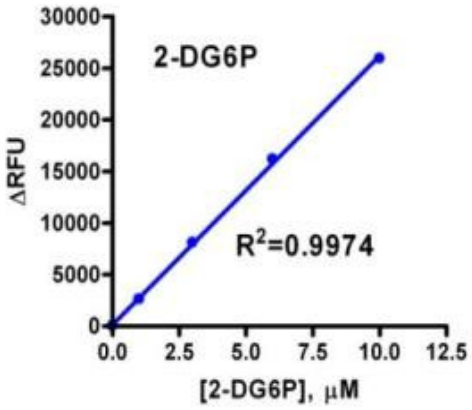
5. Calculation

Subtract the RFU of the blank from the RFU of the standards and plot the result (ΔRFU) versus the 2-DG6P concentration of the standards (µM). Determine the slope by linear regression and calculate the concentration of 2-DG6P in the samples.

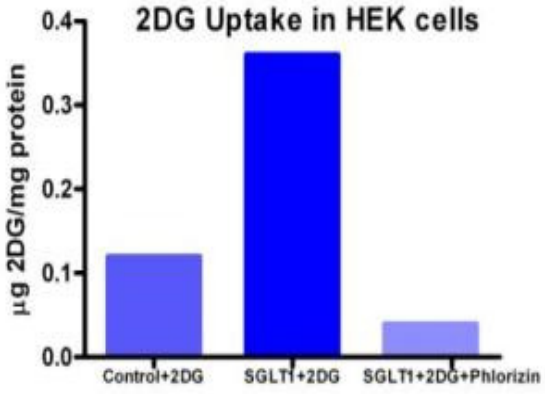
$$[\text{2-DG6P}] = \frac{RFU_{sample} - RFU_{blank}}{\text{Slope}} \times n \text{ (pmole/µL; µM)}$$

RFU_{sample} and RFU_{blank} are related fluorescence intensity of the treated and untreated sample, *n* is the dilution factor.

Typical Standard Curve



2-DG6P Standard Curve



2DG uptake was measured in SGLT1-transfected HEK cells in the presence and absence of phlorizin (100 µM)

Always run your own standard curves for calculation of results.

Sensitivity and Limit of Detection

The Limit of Detection was determined as 0.05 µM, and linear detection range up to 10 µM in 96-well plate fluorometric assay. Sensitivity was determined as 0.05 µM. Samples with values above 10 µM should be dilute with dH_2O or assay buffer, re-assayed, and multiply results by dilution factor.

Interferents

This alkali-heating process is performed to lyse the cells and to destroy enzymes as well as endogenous NAD(P) and DNA(P)H. Results may be adversely affected if NAD(P)H and enzymes remain in the sample.

References

Yamamoto, N. et al. 2015, Current Protocols in Pharmacology. 71:12.14.1-12.14.26
Huan, Y. et al. 2013, Acta Pharmaceutica Sinica B 3:97-101
Kanwal, A. 2012, Anal. Biochem. 429:70-75-421

Related Products:

Glucose Uptake Cell-Based Assay Kit (#K958f-100)

Glucose Uptake Colorimetric Assay Kit (#K958c-100)