Glucose Uptake Colorimetric Assay (TBS2064, 100Assay, Store at -20°C)

DESCRIPTION

The glucose uptake assay is a method used to measure the uptake of glucose by cells, which is a key indicator of cellular metabolism. Insulin plays a crucial role in facilitating glucose uptake, especially after meals. Glucose transporters (GLUT proteins) are responsible for moving glucose across cell membranes. Impaired glucose uptake can lead to conditions like insulin resistance, diabetes, heart disease, and Alzheimer's disease. Glucose uptake assays are widely used to quantify the transport and uptake of glucose in cells.

Tribioscience's Glucose Uptake Colorimetric Assay Kit provides a flexible, accurate, sensitive, and time-saving approach for detecting glucose uptake in a wide variety of samples. In this assay, 2-deoxyglucose (2-DG) is used as a glucose analog and moved into cell, then metabolized to 2-deoxyglucose 6-phosphate (2-DG6P) in cell. 2-DG6P can be measured by enzyme colorimetric reactions. The color change is proportional to the uptake of 2-DG, which can be detected at 460 nm.

FEATURES

- **Flexible:** Suitable for 96-well or 384-well plate.
- **Accuracy**: Absorbance measurement is proportional to the 2DG6P concentration.
- **Sensitive:** Can detect as low as 10 µM of 2-DG6P.
- **Non-radioactive**

Kit Components and Storage for 100 Assays

Part Name	Part Size	Part Number
Assay Buffer	10 mL	TBS2064A
Enzyme1	1.1 mL	TBS2064B
Enzyme2	0.55 mL	TBS2064C
Co-substrate	1.1 mL	TBS2064D
Probe	1.2 mL	TBS2064E
Cofactor	0.55 mL	TBS2064F
Substrate	1 mL	TBS2064G
Standard	0.1 mL	TBS2076H

APPLICATIONS

- Glucose uptake in different samples like cells and tissues.

Sample Preparation

A. Culture, Starve and Treat Cells

1. Seed 100 µL of 1-10 x10^3 adherent cells or 1-5x10^4 suspend cells into each well of 96-well culture plate. Incubate overnight in normal cell culture condition (different cells adjust density).
2. Starved cells with 0.5% serum and lower glucose medium 6 hours to overnight, then stimulate with or without 1 µM insulin or any treatments for 60 minutes, then washed cells with 150 µL of 1x PBS twice.
3. Add 10 µl of 2-DG substrate and 90 µl of glucose free cell medium to each well. Incubate for 30 minutes or desired time.
4. Washed cells by 150 µL of 1x PBS twice. Cells (or Tissues) are homogenized in 3 volumes of the assay Buffer.
5. Centrifuge the samples at 2000xg for 10 min at 4°C.
6. Take the supernatant to the new tube on the ice as samples.

Assay Procedures

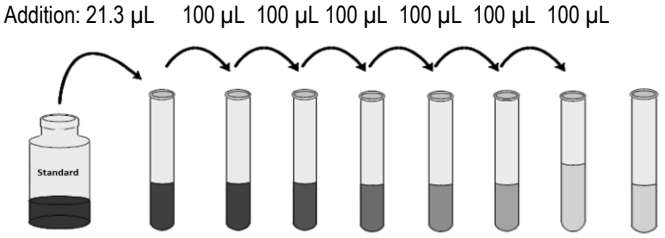
1. Standard Preparation:

1.1 Label test tubes as #1 through #8. Pipet 178.7 µL of 1x Assay Buffer into tube #1, and 100 µL into tubes #2 to #8 as Fig, 1. diagram.

1.2 Add 21.3 µL of the 2-DG6P Standard stock (6mM) to tube #1 and mix to make standard concentration of 640 µM.

1.3 Make 2x serial dilutions of the standard using the Tube#1(640 µM standard solution) from Tube #2 through #7 with sequential transfer of 100 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 640, 320, 160, 80, 40, 20 and 10 µM. Tube# 8 is Standard 8 as blank (0 µM).

Fig.1 Diagram for standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	178.7	100	100	100	100	100	100	100
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	21.3	100	100	100	100	100	100	0
Conc (µM)	640	320	160	80	40	20	10	0

2. Loading samples:
Add 50 µL Standard, sample, and control to each well. (Note: recommend running a pilot study to determine the optimal concentration of sample within the assay standard curve range).

3. Preparation of reaction mix: mixing the below reagents for one well, total volume 50 µL.

Reaction Mix	For Sample	For Std
Assay Buffer	10 µL	25 µL
Enzyme 1	10 µL	0 µL
Enzyme 2	5 µL	5 µL
Co-substrate	10 µL	10 µL
Cofactor	5 µL	0 µL
Probe	10 µL	10 µL

4. Add 50 µL of reaction mix to appropriate well containing the standards, controls, and samples.

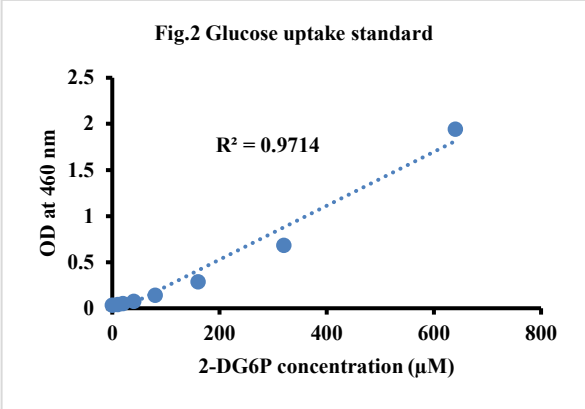
5. Incubate at room temperature for 30-60 minutes at room temperature, protected from light with gently shaking.

6. Measure OD460 nm.

7. Calculate the glucose uptake with the 2-DG6P standard curve.
Typical 2-DG6P standard curve: Y=AX + B
Y is ΔOD460: the OD460 subtract the blank.
X is the 2-DG6P concentration: μM.
A is the slope and B is the Y-intercept

2-DG6P (μM) = (ΔOD460nm - B) * N /A
ΔOD460 is the OD460 subtract the blank.
N: Dilution Factor.

Typical standard curve for example only as shown Fig 2:



RELATED PRODUCTS
6-Phosphogluconate Colorimetric Assay (TBS2089C)
6-Phosphogluconate Dehydrogenase Activity Colorimetric Assay (TBS2093C)
Glucose-6-phosphate dehydrogenase Activity Colorimetric Assay (TBS2102C)
Glucose Quantity Assay (TBS2087)
Glucose Oxidate Activity Assay (TBS2088)
Resazurin Cell Viability (TBS2001)
LDH Cytotoxicity Assay (TBS2002)
Catalase Assay (TBS2006)
ATP Colorimetric/ Fluorometric Assay (TBS 2010)
ADP Colorimetric/Fluorometric Assay (TBS3020)
CCK-8 Cell Viability Assay (TBS2022)
Caspase-3 Colorimetric Assay (TBS2030)
Cytochrome c Reductase Activity Assay (TBS2116)
AOPI Viability Assay for Flow Cytometry (TBS2069

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