Technical Bulletin

Glucose Uptake Assay Kit

Catalogue number MAK542

Product Description

Glucose transport systems are responsible for transporting glucose across cell membranes. Measuring uptake of 2-deoxyglucose (2-DG), a glucose analog, in tissues and cells is widely accepted as a reliable method to estimate the amount of glucose uptake and to investigate the regulation of glucose metabolism and mechanism of insulin resistance.

The Glucose Uptake Assay Kit provides a sensitive and non-radioactive assay in tissues or cultured cells. In this assay, 2-DG is taken up by glucose transporters and metabolized to 2-DG-6-phosphate (2-DG6P). The non-metabolizable 2-DG6P accumulates in the cells and is proportional to glucose uptake by cells. The accumulated 2-DG6P is enzymatically oxidized and generates NADPH, which is specifically monitored by a chromogenic NADPH sensor. The signal can be read by a Spectrophotometric plate reader by reading the OD ratio at wavelength 570 nm to 610 nm.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- 2-Deoxyglucose (2-DG, 10mM) 1 mL
  Catalogue Number MAK542A
- Glucose Uptake Buffer 10 mL
  Catalogue Number MAK542B
- Acidic Lysis Buffer 2.5 mL
  Catalogue Number MAK542C
- Neutralization Buffer 2.5 mL
  Catalogue Number MAK542D
- Enzyme Probe 1 Vial
  Catalogue Number MAK542E
- Assay Buffer 5 mL
  Catalogue Number MAK542F
- NADP 1 Vial
  Catalogue Number MAK542G
- 5x KRPH Buffer 20 mL
  Catalogue Number MAK542H

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Spectrophotometric multiwell plate reader.
- Clear-bottom 96-well cell culture microplate.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Preparation of KRPH Buffer Stock Solutions

Add 20 mL of KRPH Buffer (5X) to 80 mL of purified water and mix well.

Note: 50 mL volume of 1× KRPH Buffer is enough for one 96-well plate. Prepare the needed volume proportionally. Store the unused stock solution at 4 °C or -20 °C. Avoid repeated freeze-thaw cycles.

Preparation of Working Solutions

NADP Working Solution: Add 100 µL of purified water into the vial of NADP and mix well.

Enzyme Probe Working Solution: Add 5 mL of Assay Buffer into the vial of Enzyme Probe and mix well.

2-DG Uptake Assay Working Solution: Add 100 µL of NADP Working Solution into the Enzyme Probe Working Solution and mix well.

Sample Preparation

Note: The following cell treatment procedure is presented as a guideline only. This protocol can be used as guidelines to culture 3T3-L1 adipocytes for 2-DG uptake. For other cell types, optimal incubation and treatment procedures may vary.

1. Plate cells in growth medium at 50,000-80,000 cells/well/100 µL in a 96-well black wall/clear bottom cell culture Poly-D lysine plate for 4-6 hours before experiment.
2. Remove the cell plate from the incubator, aspirate the medium from the wells, and deprive the cells with 100 µL/well serum-free medium. Incubate the cells at 37 °C, 5% CO_2 incubator for 6 hours to overnight.
3. Remove the plate from the incubator, aspirate the medium from the wells, and gently wash the cells twice with 100 µL/well 1× KRPH buffer.
4. Add 90 µL/well Glucose Uptake Buffer and incubate the cells at 37 °C, 5% CO_2 incubator for 1 hour.
5. Stimulate with or without insulin or test compound for 20 minutes. Add 10 µL/well of the 10× insulin solution to a final concentration of 1 µM or 10× compound solution of test. Add 10 µL insulin vehicle buffer or compound vehicle buffer to the untreated wells as control, and incubate at 37 °C, 5% CO_2 incubator for 20 minutes.
6. For glucose uptake inhibition study, add 10× Phloretin to a final concentration of 200 µM or inhibitors of test, and incubate at 37 °C, 5% CO_2 for 2-5 minutes.

   Note: 10 µL inhibitor vehicle buffer is suggested to be added to both the insulin treated and untreated wells as control. Phloretin treated cells can be used as positive control.

Assay Reaction

1. Add 10 µL/well 2-DG solution to each well, and incubate at 37 °C, 5% CO_2 incubator for 20-40 mins. For negative controls, leave some wells untreated with insulin, inhibitor, and 2-DG.
2. After treatment, remove solution in each well and gently wash cells 3 times, 100 µL/well with KRPH Buffer to remove the extra 2-DG from the solution. Then remove the KRPH Buffer from the wells.
3. Add 25 µL of Acidic Lysis Buffer to each well and incubate at 37 °C for 20 minutes to lyse the cells. The 2-DG Uptake Assay Working Solution may be prepared at this time.
4. Add 25 µL of Neutralization Buffer to each well, mix thoroughly, leave at room temperature for 5-10 minutes to neutralize the cell lysate.
5. Add 50 µL of 2-DG Uptake Assay Working Solution to each well of 2-DG6P standard (Not provided) and cell lysate.
6. Incubate the reaction mixture at room temperature for 30 minutes to 2 hours, protected from light.

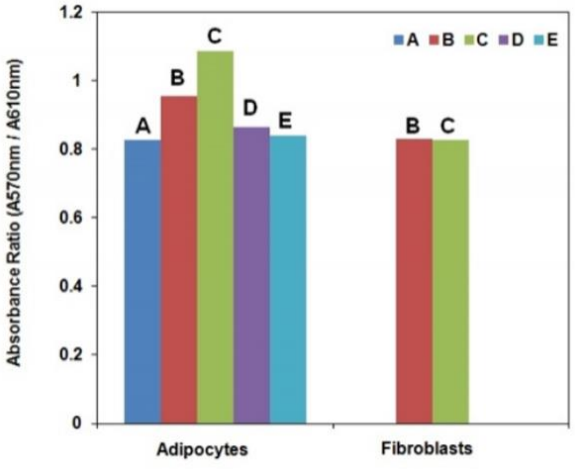
Measurement

Monitor the absorbance increase with an absorbance plate reader with path check on at OD of 570 nm to 610 nm.

Figure 1.

Example of measurement of 2DG uptake in differentiated 3T3-L1 adipocytes and 3T3-L1 fibroblasts.

A: Negative Control, no insulin, no 2-DG treatment.
B: 2-DG uptake in the absence of insulin.
C: 2-DG uptake in the presence of 1 µM insulin.
D: 2-DG uptake in the presence of 1 µM insulin and 200 µM phloretin.
E: 2-DG uptake in the presence of insulin 1 µM and 5 µM D-Glucose.



Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

mak542pis Rev 9/25