Technical Bulletin

Glucose Uptake Assay Kit

Catalogue number MAK489

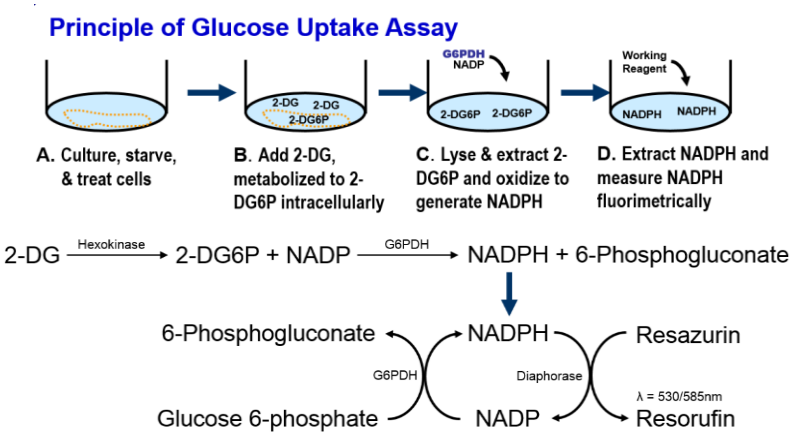
Product Description

Glucose uptake has a variety of methods and transporters and depends upon the metabolic demand of the cell type and availability of glucose. There are over ten different facilitated diffusion glucose transporters which transport glucose down its concentration gradient without ATP hydrolysis. In the kidneys, secondary active transport is used to uptake glucose against its concentration gradient to ensure that very little glucose is excreted in urine.

The Glucose Uptake Assay Kit is a fluorescent cell-based assay that uses 2-deoxyglucose (2-DG), a widely used glucose analog which is taken up by glucose transporters and metabolized by endogenous hexokinase into 2-deoxyglucose 6-phosphate (2-DG6P).

2-DG6P accumulates intracellularly because it is not a suitable substrate for phosphoglucose isomerase, the next step in glycolysis. The cells are lysed, and excess NADP and glucose 6-phosphate dehydrogenase (G6PDH) are added to metabolize the 2-DG6P and generate a molar equivalent amount of NADPH. The NADPH is then measured using a G6PDH recycling reaction to amplify the signal and generate a fluorescent signal measurable at λ_{Ex} = 530 nm/λ_{Em} = 585 nm proportional to the concentration of 2-DG6P.

The linear detection range of the kit is 0.1 – 5 µM 2-DG6P. The kit is suitable for the determination of glucose uptake in whole cells and evaluation of effects of ligands or drugs on glucose transport.



Components

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

- Assay Buffer — 10 mL
  Catalogue Number MAK489A
- G6P Reagent — 1.5 mL
  Catalogue Number MAK489B
- Enzyme A — 120 µL
  Catalogue Number MAK489C
- Enzyme B — 120 µL
  Catalogue Number MAK489D
- NADP — 120 µL
  Catalogue Number MAK489E
- 2-DG Substrate — 1.2 mL
  Catalogue Number MAK489F
- Probe — 750 µL
  Catalogue Number MAK489G
- NADP Extraction Buffer — 12 mL
  Catalogue Number MAK489H
- NADPH Extraction Buffer — 12 mL
  Catalogue Number MAK489I
- 2-DG6P Standard (5 mM) — 120 µL
  Catalogue Number MAK489J

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Fluorescent multiwell plate reader
- 96-well cell culture plate
- Cell culture incubator capable of 37 °C and 80 °C
- 96-Well Black Polystyrene Microplate (Catalogue Number CLS3603 or equivalent)
- Triton® X-100 (Catalogue Number T8787 or equivalent)
- Phosphate Buffered Saline (Catalogue Number P4417 or equivalent)

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 all components to room temperature prior to use.

To avoid cross-contamination, change pipette tips between additions of each reagent or sample. The use of a multi-channel pipette is recommended. Use separate reservoirs for each reagent.

Procedure

It is recommended that Samples be assayed in triplicate or higher.

Culture, Starve, and Treat Cells

1. Seed 100 µL of 1-10 × 10^3 adherent cells (or 1-5 × 10^4 suspension cells) into each well of a 96-well cell culture plate. Incubate for 4 hours or overnight at 37 °C in a cell culture incubator.

   Note: The cell number to be used depends on cell size and metabolic demand of glucose.

2. Incubate the cells with serum-free media for 4 hours or overnight at 37 °C to increase their glucose demand.
3. Starve the cells in glucose-free and serum-free media for 40 minutes at 37 °C.
4. Add any drugs or experimental treatments to the starvation media at this step if desired. Make sure to include a control cell culture group without any experimental conditions.

Addition of 2-Deoxyglucose

1. Add 10 µL of 2-DG Substrate to each Sample well.
2. Incubate for 20 minutes or desired time.
3. Remove the media.
4. Wash the cells three times with 150 µL of ice-cold PBS to remove excess 2-DG. Each wash should be performed for 30 seconds without shaking. Ensure that cells are not disturbed.

Standard Curve Preparation

1. Prepare 5 µM 2-DG6P by mixing 5 µL of the 5 mM 2-DG6P and 4995 µL of purified water.
2. Prepare 2-DG6P standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1
Preparation of 2-DG6P Standards

Well	5 µM 2-DG6P	Purified Water	2-DG6P (µM)
1	100 µL	-	5.0
2	60 µL	40 µL	3.0
3	30 µL	70 µL	1.5
4	-	100µL	0

3. Transfer 50 µL of Standards into separate wells of the cell culture plate.

Lyse and Extract 2-Deoxyglucose 6-Phosphate

1. Prepare Lysis Buffer by adding 1% Triton® X-100 in NADP Extraction Buffer. Prepare 55 µL of Lysis Buffer per well.
2. Transfer 50 µL of Lysis Buffer to each Sample well. Do not add to Standard wells.
3. Place the cell culture plate on a rotary shaker for 5 minutes.
4. Incubate the plate at 80 °C for 10 minutes.
5. Add 50 µL of NADPH Extraction Buffer to each Sample well.
6. Add 50 µL of purified water to each Standard well.
7. Cool the plate in a -20 °C freezer for 5 minutes followed by 10 minutes at room temperature. Alternatively, cool the plate at room temperature for ~30 minutes.

Preparation of Working Reagent 1

1. Mix enough reagents for the number of assays to be performed. For each Standard and Sample well, prepare 12 µL of Working Reagent 1 according to Table 2.

Table 2.
Preparation of Working Reagent 1

Reagent	Volume
Assay Buffer	10 µL
Enzyme A	1 µL
NADP	1 µL

2. Add 10 µL of Working Reagent 1 to all wells and incubate at 37 °C for 60 minutes.

Extract NADPH

1. Add 50 µL of NADPH Extraction Buffer to all wells and incubate at 80 °C for 15 minutes.
2. Add 50 µL of NADP Extraction Buffer to all wells and cool the plate in a -20 °C freezer for 5 minutes. Alternatively, the plate can be cooled in a refrigerator or on the bench top.
3. Transfer 50 µL of Sample and Standard from each well in the cell culture plate into separate wells of a black 96-well microplate.

Preparation of Working Reagent 2

Note: Prepare Working Reagent 2 fresh just prior to use.

1. Dilute Enzyme A 40-fold by mixing 3 µL of Enzyme A with 117 µL of Assay Buffer. Diluted Enzyme A is stable for 1 month when stored at -20 °C.
2. Mix enough reagent for the number of assays to be performed. For each Standard and Sample well, prepare 62 µL of Working Reagent 2 according to Table 3.

Table 3
Preparation of Working Reagent 2

Reagent	Volume
Assay Buffer	45 µL
Diluted Enzyme A	1 µL
Enzyme B	1 µL
G6P Reagent	10 µL
Probe	5 µL

3. Add 50 µL of Working Reagent 2 to all wells.

Measurement

Immediately read the fluorescence intensity of the plate at λ_{Ex} = 530 nm/λ_{Em} = 585 nm (F_0) and again at 20 minutes (F_{20}).

Alternatively, monitor the fluorescence intensity of the plate for 20 minutes in kinetic mode and record the 0-minute and 20-minute readings.

If the fluorescent intensity for any Sample is higher than the fluorescence intensity of the 5 µM Standard, dilute the Sample in purified water and repeat assay beginning at Step 3 of the Extract NADPH section. Multiply the results by the dilution factor (DF).

Results

1. Calculate ΔF by subtracting F_0 from F_{20} for each Standard and Sample. If duplicate or triplicate Samples were performed, calculate the mean ΔF for each Sample.
2. Plot the Standard ΔF values versus Standard concentration and determine the slope of the Standard curve.
3. Calculate the concentration of 2-DG6P as follows:

$$\text{2-DG6P } (\mu M) = \frac{\Delta F_{Sample} - \Delta F_{Blank}}{\text{Slope } (\mu M^{-1})} \times DF$$

where:

ΔF_{Sample} = Fluorescence intensity reading of Sample

ΔF_{Blank} = Fluorescence intensity reading of Blank

DF = Sample dilution factor
(DF = 1 for undiluted Samples)

Figure 1.
PANC-1 cell titration in the absence and presence of 1 mM phloretin. Cells were seeded, starved, and treated according to the protocol.

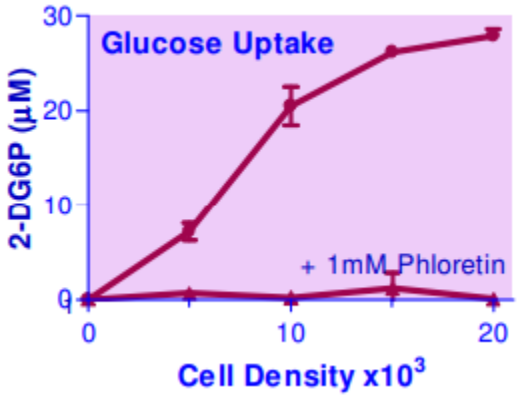
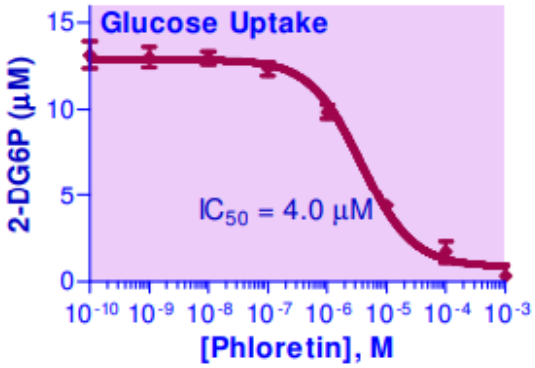


Figure 2.

Glucose transport inhibition curve with phloretin. PANC-1 cells were seeded at 10,000 cells per well



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

mak489pis Rev 12/23