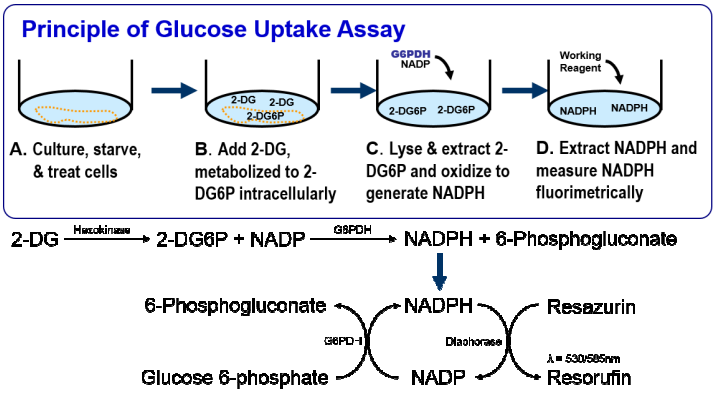
EnzyFluo™ Glucose Uptake Assay Kit (EFGU-100)

Fluorimetric Cell-Based Assay for Glucose Uptake

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 transports 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.

BioAssay Systems' fluorescent cell-based glucose uptake assay uses 2-deoxyglucose (2-DG), a widely used glucose analog because it can be 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) is added to metabolize 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 measureable at $\lambda_{ex/em}$ = 530/585 nm proportional to the concentration of 2-DG6P.



KEY FEATURES

Safe. No radioactive material is used.

Sensitive and Accurate. Detection limit of 0.1 µM and linearity up to 5 µM 2-DG6P.

Simple and Convenient. Can be automated as a medium throughput assay for glucose transport in cells.

APPLICATIONS

Determination of glucose uptake in whole cells.
Evaluation of effects of ligands or drugs on glucose transport.

KIT CONTENTS

Assay Buffer:	10 mL	**G6P Reagent:**	1.5 mL
Enzyme A:	120 µL	**Enzyme B:**	120 µL
2-DG Substrate:	1.2 mL	**NADP:**	120 µL
2-DG6P Standard:	120 µL	**Probe:**	750 µL

NADP/NADPH Extraction Buffers: each 12 mL

Storage conditions: store all reagents at -20°C. Shelf life of 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURE

Important:

1. To avoid cross-contamination, change pipette tips between additions of each reagent or sample. We recommend the use of a multi-channel pipette. Use separate reservoirs for each reagent.
2. It is recommended that samples be assayed in triplicate or higher.

A. 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 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 low glucose and either 0.2% serum or serum-less media for 4 hours or overnight to increase their glucose demand.
3. Gently wash the cells two (2) times with 150 µL of 37°C PBS to remove all traces of glucose.
4. Starve the cells in glucose-less and serum-less media for 40 minutes. Add any drugs or experimental treatments to the starvation media at this step if desired. Make sure to include a control group without any experimental conditions.

B. Add 2-Deoxyglucose

1. Add 10 µL of 2-DG substrate to each well. Incubate for 20 minutes or desired time.
2. Remove the media. Then wash the cells three (3) times with 150 µL of ice-cold PBS to remove excess 2-DG. Each wash should be performed for 30 seconds without shaking, try not to disturb the cells.

C. Lyse and Extract 2-Deoxyglucose 6-phosphate

1. Prepare Lysis Buffer by adding 1% Triton X-100 to NADP Extraction Buffer. Prepare enough for 55 µL per well.
2. Transfer 50 µL of Lysis Buffer to each well with cells, place the plate on a rotary shaker for 5 minutes, and then incubate the plate at 80°C for 10 minutes.
3. *Standard Curve.* Prepare 5 µM 2-DG6P Premix by mixing 5 µL 5 mM Standard and 4995 µL distilled water. Dilute standard as follows.

No	Premix + H_2O	2-DG6P (µM)
1	100 µL + 0 µL	5.0
2	60 µL + 40 µL	3.0
3	30 µL + 70 µL	1.5
4	0 µL + 100 µL	0

   Transfer 50 µL standards into separate wells of the cell plate.
4. Add 50 µL of NADPH extraction buffer to each well with cells, and 50 µL of dH_2O to the standard wells. Cool the plate in a -20°C freezer for 5 minutes followed by 10 min on the bench top (alternatively you may also cool the plate to room temperature on the bench top (~30 min)).
5. *Working Reagent (WR1) Preparation.* For each reaction well, prepare WR1 by mixing 10 µL Assay Buffer, 1 µL Enzyme A, 1 µL NADP. Add 10 µL of WR1 to all wells and incubate at 37°C for 60 minutes.

D. Extract and Measure 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 you may also leave the plate in a refrigerator or on the bench top).
3. Transfer 50 µL of sample and standard from each well in the cell plate into separate wells in a black 96-well plate.
4. *Working Reagent (WR2) Preparation*. For the following reaction, dilute Enzyme A 40-fold by mixing 3 µL Enzyme A with 117 µL Assay Buffer. Diluted Enzyme A is stable for 1 month when stored at -20ºC.

   For each reaction well, prepare *WR2* by mixing 45 µL Assay Buffer, 1 µL diluted Enzyme A, 1 µL Enzyme B, 10 µL G6P Reagent and 5 µL Probe. Fresh reconstitution is recommended.
5. Transfer 50 µL of WR2 into each well. Read the plate at $\lambda_{ex/em}$ = 530/585 nm for 20 minutes. Use data from time zero and 20 minutes (F_0) and (F_{20}).

   Note: If fluorescent signal for any sample is higher than the fluorescence of the 5 uM standard, dilute the sample in dH_2O and repeat Steps 3-5 in D. Extract and Measure NADPH. *Multiply the results by the dilution factor.*

CALCULATION

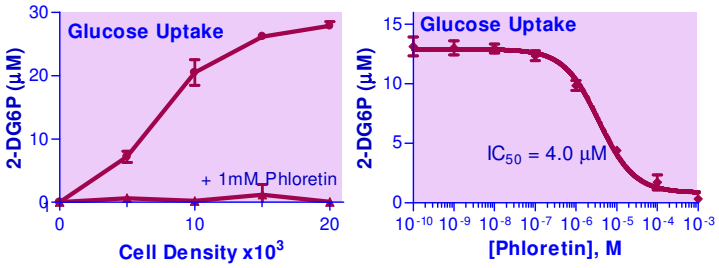
First compute the ΔF for each standard and sample by subtracting F_0 from F_{20}. If duplicate or triplicate samples were performed, calculate the mean ΔF intensities for the Sample wells. Plot the standard ΔF's and determine the slope. The concentration of 2-DG6P is calculated as follows:

$$[\text{2-DG6P}] = \frac{\Delta F_{Sample} - \Delta F_{Blank}}{\text{Slope}} \times n \quad (\mu M)$$

where ΔF_{Sample} is the mean ΔF of the samples, and ΔF_{Blank} is the ΔF of the water, standard #4 blank. Slope is the slope of the standard curve and n is the dilution factor.

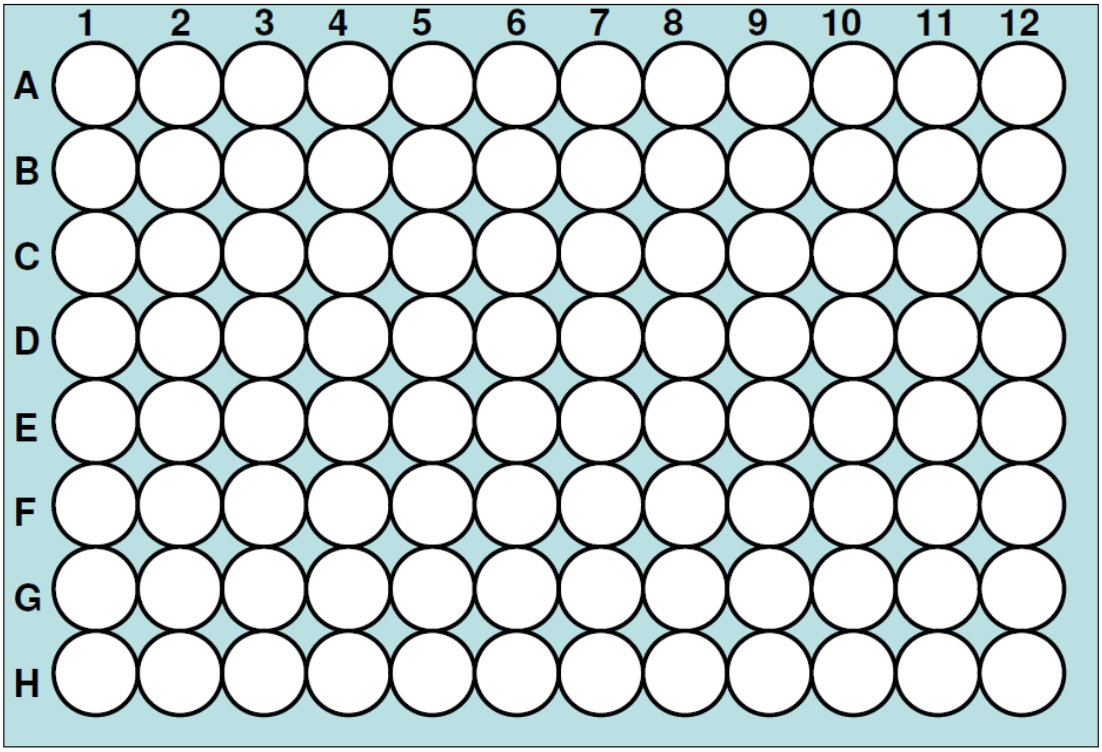
MATERIALS REQUIRED BUT NOT PROVIDED

Triton X-100 (Sigma, cat # T8787); Phosphate Buffered Saline (Sigma, cat # P4417; can also be made yourself if desired); black cell culture 96-well plate: available separately at BioAssay System (cat# P96BCC) or at Sigma (CLS3603); deionized or distilled water; pipetting devices; cell culture incubators; centrifuge tubes; fluorescence plate reader capable of reading at $\lambda_{ex/em}$ = 530/585 nm.



Glucose Transport Assay in PANC-1 Cells. Cells were seeded, starved, and treated according to protocol. *Left:* PANC-1 cell titration in the absence and presence of 1 mM phloretin. *Right:* glucose transport inhibition curve with phloretin. PANC-1 cells were seeded at 10,000 cells per well.

PUBLICATIONS

1. Liu, Meihan, et al (2019). Activation of AMPK by metformin promotes renal cancer cell proliferation under glucose deprivation through its interaction with PKM2. International Journal of Biological Sciences 15.3: 617-627.
2. Park, Seong-Hoon, et al(2016). SIRT2-mediated deacetylation and tetramerization of pyruvate kinase directs glycolysis and tumor growth. Cancer research 76.13: 3802-3812.
3. Tunduguru, Ragadeepthi (2016). Regulation of skeletal muscle insulin sensitivity by PAK1. Dissertation. Assay: Glucose uptake in rat cells.

96-Well Assay Plate Template