Glucose Uptake Assay Kit (Fluorometric)

Glucose Uptake Assay Kit (Fluorometric) is a detection kit for the quantification of Glucose Uptake in cell culture.

Catalog number: ARG82163

Package: 100 tests

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INTRODUCTION

Glucose is a simple sugar with the molecular formula $C_6H_{12}O_6$. Glucose is the most abundant monosaccharide, a subcategory of carbohydrates. Glucose is mainly made by plants and most algae during photosynthesis from water and carbon dioxide, using energy from sunlight, where it is used to make cellulose in cell walls, the most abundant carbohydrate in the world.

In energy metabolism, glucose is the most important source of energy in all organisms. Glucose for metabolism is stored as a polymer, in plants mainly as starch and amylopectin, and in animals as glycogen. Glucose circulates in the blood of animals as blood sugar. The naturally occurring form of glucose is d-glucose, while l-glucose is produced synthetically in comparatively small amounts and is of lesser importance. Glucose is a monosaccharide containing six carbon atoms and an aldehyde group, and is therefore an aldohexose. The glucose molecule can exist in an open-chain (acyclic) as well as ring (cyclic) form. Glucose is naturally occurring and is found in fruits and other parts of plants in its free state. In animals, glucose is released from the breakdown of glycogen in a process known as glycogenolysis. [Provide by Wikipedia: Glucose]

PRINCIPLE OF THE ASSAY

This Glucose Uptake Assay Kit (Fluorometric) is a simple fluorometric assay. This 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 λex/em = 530/585 nm proportional to the concentration of 2-DG6P.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all components at -20°C. Shelf life of 6 months after receipt.

Component	Quantity	Storage information
2-DG6P Standard (5 mM)	120 µL	-20°C
2-DG Substrate	1.2 mL	-20°C
NADP	120 µL	-20°C
Enzyme A	120 µL	-20°C
Enzyme B	120 µL	-20°C
G6P Reagent	1.5 mL	-20°C
Assay Buffer	10 mL	-20°C
Probe	750 µL	-20°C
NADP Extraction Buffers	12 mL	-20°C
NADPH Extraction Buffers	12 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm.
- Centrifuge
- Cell culture incubator
- Culture medium
- Triton X-100
- 1X PBS
- Deionized or distilled water
- Black 96-well plate
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates or higher.
- Change pipette tips between the addition of different reagent or samples. We recommend the use of a multi-channel pipette. Use separate reservoirs for each reagent.

SAMPLE COLLECTION & STORAGE INFORMATION

Adherent cells or suspension cells. The cell number to be used depends on cell size and metabolic demand of glucose.

REAGENT PREPARATION

- **Lysis Buffer:** Add Triton X-100 to NADP Extraction Buffer to a final Triton X-100 concentration of 1%. Prepare enough Lysis Buffer for 55 µL per well.
- **Working Reagent 1:** For each reaction well, mixing as follows: (10 µL per well is needed)

  10 µL of Assay Buffer,

  1 µL of Enzyme A,

  1 µL of NADP.
- **Working Reagent 2:** Dilute Enzyme A for 40X dilution with Assay Buffer (add 3 µL of Enzyme A with 117 µL of Assay Buffer, this diluted Enzyme A is stable for 1 month when stored at -20°C).

  For each reaction well, mixing as follows: (50 µL per well is needed)

  45 µL of Assay Buffer,

  1 µL of **40X diluted** Enzyme A,

  1 µL Enzyme of B,

  10 µL of G6P Reagent,

  5 µL of Probe.

  Fresh reconstitution is recommended.
- **Standards:** Prepare 5 µM of 2-DG6P Premix by mixing 5 µL of 5 mM Standard stock and 4995 µL of distilled water. Dilute standards as follows.

Standard tube	2-DG6P (µM)	Distilled water (µL)	Standard premix 5 µM (µL)
S1	5.0	0	150
S2	3.0	60	90
S3	1.5	105	45
S4	0	150	0

ASSAY PROCEDURE

Prior to the assay, equilibrate all components to room temperature. Briefly centrifuge tubes before opening.

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-less media** for **4 hours or overnight** to increase their glucose demand.
3. 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.

Add 2-Deoxyglucose

1. Add **10 µL** of **2-DG substrate** to each well. Incubate for **20 minutes** or **desired time**. (2-DG metabolized to 2-DG6P intracellularly)
2. Remove the medium. Then wash the cells **3 times** with **150 µL** of **1X cold PBS** to remove excess 2-DG. Each wash should be performed for **30 seconds** without shaking, try not to disturb the cells.

Lyse and Extract 2-Deoxyglucose 6-phosphate

	Standard wells	Sample wells (with cells)
Standards	50 µL	-
Lysis Buffer	-	50 µL
Place the plate on a rotary shaker for **5 minutes**, and then incubate the plate for **10 minutes** at **80°C**.		
NADPH Extraction Buffer	-	50 µL
Distilled water	50 µL	-
Cool the plate in a **-20°C** freezer for **5 minutes** followed by **10 minutes** on the bench top (alternatively user may also cool the plate to room temperature on the bench top for about 30 minutes).		
Working Reagent 1	10 µL	10 µL
Incubate for **60 minutes** at **37°C**.		

Extract and Measure NADPH

	Standard wells	Sample wells (with cells)
NADPH Extraction Buffer	50 µL	50 µL
Incubate for **15 minutes** at **80°C**.		
NADP Extraction Buffer	50 µL	50 µL
Cool the plate in a **-20°C** freezer for **5 minutes** (alternatively user may also leave the plate in a refrigerator or on the bench top).		
Transfer **50 µL** of **samples** and **Standards** from each well in the cell culture plate into separate wells in a **black 96-well microplate**. (**Note:** If fluorescent signal for any sample is higher than the fluorescence of the 5 µM standard, dilute the sample in distilled water and repeat assay from this step. Transfer **50 µL** of **diluted samples** and **Standards** in a **black 96-well microplate.** And then add Working Reagent 2 and read the fluorescence. Multiply the results by the dilution factor.)		
Working Reagent 2	50 µL	50 µL
Read the plate at 0 and 20 minutes with a fluorescence microplate reader using **excitation 530 nm filter** and **emission 585 nm filter.**		

CALCULATION OF RESULTS

1. 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:

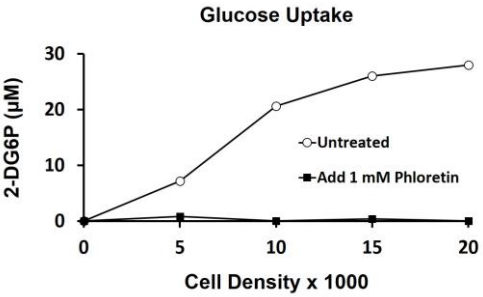
   2-DG6P (µM) = [(ΔF_{Sample}- ΔF_{Blank}) / Slope] x n

   Note:

   - ΔF_{Sample} : the ΔF of sample
   - ΔF_{Blank}: the ΔF of Standard 4 (S4) with Distilled water only as blank.
   - Slope is the slope of the standard curve and n is the dilution factor.

EXAMPLE OF RESULT

The following figures demonstrate typical results with the Glucose Uptake Assay Kit (Fluorometric). One should use the data below for reference only. This data should not be used to interpret actual results.



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

QUALITY ASSURANCE

Sensitivity

0.1 µM

Assay Range

0.1 µM to 5 µM 2-DG6P