

ab287845 – 2-NBDG Glucose Uptake Assay Kit (Cell-Based)

For imaging and accurate measurement of glucose uptake in cultured cells.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<https://www.abcam.com/ab287845>

Introduction

Glucose is a ubiquitous energy source in most organisms and plays a pivotal role in cellular metabolisms and homeostasis. Cancer cells exhibit increased glucose uptake to support their high proliferation rate. 2-NBDG (2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl) amino]-D-glucose) is a fluorescent deoxyglucose analog that can be taken up by cells through glucose transporters. However, 2-NBDG cannot be fully utilized in glycolysis because of its modification and thus accumulates inside the cells. Fluorescence generated by this fluorescent glucose analog is proportional to glucose uptake by the cells and can be used to measure glucose uptake using fluorescent microscopy and flow cytometry. To validate the assay, the kit includes phloretin, a natural phenol that inhibits glucose uptake. This easy to use non-radioactive kit allows imaging and accurate measurement of glucose uptake in cultured cells in response to insulin, growth factors etc.

Applications

- Measurement of glucose uptake in response to insulin, growth factors, cytokines, mitogens, and nutrients, etc.
- Analysis of glucose metabolism and cell signalling in various cell types
- Screening anti-diabetic compound

Sample Type

- Adherent or suspension cells

Storage and Stability

Store kit at -20°C, protected from light.

Materials Supplied

| Item | Quantity | Storage Condition |
|-------------------------|----------|-------------------|
| Buffer I | 1.8 ml | -20°C |
| 2-NBDG Reagent | 200 µl | -20°C |
| Glucose Uptake Enhancer | 1 ml | -20°C |
| 100X Phloretin | 75 µl | -20°C |

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Cell culture medium
- PBS
- 24 or 12-well tissue culture plate
- Fluorescence microscope
- Flow cytometer with excitation filter at 488 nm wavelength

Reagent Preparation

- Briefly centrifuge small vials prior to opening.
- Read entire protocol before performing the assay.

Buffer I: Buffer I is provided as a 50X stock solution. Prior to use, dilute to 1X by mixing with PBS at a 1:50 ratio (for example, mix 0.5 mL of the 50X buffer I concentrate with 24.5 mL of PBS to produce 25 mL of 1X Buffer I. Unused 1X Buffer I may be stored at 4°C for up to one week.

2-NBDG Reagent: Aliquot and store at -20°C. For consistent results, avoid repeated freeze/thaw.

Assay Protocol

The protocol described below is for 24-well tissue culture plate. Reagents, buffer, and the number of cells to be seeded should be adjusted for different size culture plates.

Sample preparation:

1. Seed adherent cells (2-5x10⁴ cells/well) one day before starting the assay.
2. After 8-12 hrs, remove regular culture medium (10% FBS) and treat cells with test compound or vehicle control in 400 µl cell culture medium with 0.5% FBS.
3. Incubate cells at 37°C with 5% CO₂ for 1 hr or desired time period depending upon the test compound. To use Phloretin as a control, treat cells with 4 µl Phloretin (final concentration 1X) in 400 µl of cell culture medium with 0.5% FBS at 37°C with 5% CO₂ for 1 hr.

Δ Note: Cell seeding is not required for suspension cells. Use up to 1-2x10⁵ suspension cells/well in 400 µl of cell culture medium with 0.5% FBS to treat with test compound or vehicle control.

Reaction Mix:

- Prepare 400 µl glucose uptake mix for each well as following:

| Item: | Volume |
|--------------------------------|--------------------------------------|
| Cell culture medium (0.5% FBS) | 376 µl |
| 2-NBDG Reagent | 4 µl |
| Glucose Uptake Enhancer | 20 µl |
| Test compound or 1X Phloretin | Same conc. as in sample preparation. |

- Mix well. After the incubation, spin down the plate at 400 x g for 5 min. and carefully remove the medium without disturbing cells. Gently add glucose uptake mix to each well and incubate cells at 37°C with 5% CO₂ for 30 min.

Measurement

After incubation, collect cells from the plate and keep on ice. Wash once with 1 ml ice-cold 1X Buffer I. Spin down at 400 x g for 5 min. and resuspend cell pellet in 400 µl of 1X Buffer I. Cells are ready to be analyzed on flow cytometer (488 nm excitation laser). For flow acquisition and analysis, select the main cell population in the FSC vs SSC plot to exclude dead cells and cellular debris. Within the main cell population, mean fluorescence intensity in FL1 can be quantified and compared between cells treated with test compounds and untreated control cells.

Δ Note: Trypsin can be used to collect the adherent cells for performing this assay.

Δ Note: The assay can be used to measure and compare glucose uptake levels in various cell types.

Δ Note: To visualize the level of glucose uptake under fluorescence microscope, centrifuge plate at 400 x g for 5 min. Wash cells once with 500 µl ice-cold 1X Buffer I and replace with fresh 200 µl of 1X Buffer I. Observe cells under fluorescence microscope using blue excitation fluorescence filter (excitation range 420 nm-495 nm).

Technical Support

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