2-NBDG Glucose Uptake Assay Kit

Catalog Number KA6077

200 assays

Version: 02

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Background

Glucose metabolism, a process which converts glucose into energy, is a primary source of energy supply in most organisms. 2-NBDG [2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose], a fluorescently tagged glucose tracer, has been proven to effectively monitor glucose transportation in cells, as 2-NBDG transports into cells by the same glucose transporters (GLUTs) as glucose. Once 2-NBDG is uptaken in cells, it undergoes phosphorylation at C-6 position to give 2-NBDG-6-phosphate, which is well retained within the cells. Compared to other glucose tracers, such as 2-DG or FDG, 2-NBDG allows in situ measurements of 2-NBDG with high temporal and spatial resolution at single cell level.

Principle of the Assay

The 2-NBDG Glucose Uptake Assay Kit provides a sensitive and non-radioactive assay for measuring glucose uptake in cultured cells. In this kit, Assay Buffer I is used to enhance the uptake and retention of 2-NBDG in cells, while Assay Buffer II can improve the signal-to-background ratio of 2-NBDG in the cells. The fluorescence signal can be monitored by fluorescence microscope or flow cytometer with a 488 nm laser and 530/30 nm emission filter (FITC channel). The 2-NBDG Glucose Uptake Assay Kit is the most robust tool for monitoring glucose transporters.

General Information

Materials Supplied

List of component

Component	Amount
Component A: 2-NBDG (10 mg/mL)	100 µL
Component B: Assay Buffer I	50 mL
Component C: Assay Buffer II	20 mL

Storage Instruction

Component	Storage
Component A: 2-NBDG (10 mg/mL)	Freeze (<-15°C). Minimize light exposure.
Component B: Assay Buffer I	Freeze (<-15°C). Minimize light exposure.
Component C: Assay Buffer II	Freeze (<-15°C). Minimize light exposure.

Materials Required but Not Supplied

Instrument: Fluorescence microscope

Excitation: FITC filter

Emission: FITC filter

Recommended plate: Black wall/clear bottom

Instrument: Flow cytometer

Excitation: 488 nm laser

Emission: 530/30 nm filter

Instrument specification(s): FITC channel

Precautions for Use

For research use only.

Assay Protocol

Reagent Preparation

✓ Preparation of working solution

Add 5 µL of 2-NBDG (10 mg/mL) (Component A) to 1.5 mL of Assay Buffer I (Component B) and mix well to make 2-NBDG staining solution. Protect from light.

Note: This 2-NBDG staining solution is stable for 1 hour at room temperature. As the optimal staining conditions may vary depending on different cell types, it's recommended to determine the optimal concentration of Component A for each specific experiment.

Sample Preparation

Prepare cells

✓ Microplate Assays (96 or 384 wells)

- For adherent cells: Plate cells overnight in growth medium. Table below provides a rough guideline of cell densities per well. In general, assays which require long incubation times (2-3 days) should be plated at a lower initial cell density.

	Examples assay	96 wells	384 wells
Standard Incubation	Calcium, NAD/NADH, membrane potential	40,000 to 80,000 cells/well/100 µL	10,000 to 20,000 cells/well/25 µL
Long Incubation	Proliferation, tracking	5,000 to 10,000 cells/well/100 µL	2500 to 5000 cells/well/ 50 µL

- For non-adherent cells:
  1. Centrifuge the cells and carefully discard the supernatant (i.e., the culture medium).
  2. Re-suspend the cell pellet in cell growth medium or HHBS. Table below provides guidelines on cell densities for resuspension. In general, assay which require long incubation times (2-3 days) should be resuspended at a lower initial cell density.
  3. Transfer resuspended cells into the assay microplate.
  4. Centrifuge the microplate at 800 rpm for 2 minutes with brake off prior to experiments.

	Examples assay	96 wells	384 wells
Standard Incubation	Calcium, NAD/NADH, membrane potential	125,000 to 250,000 cells/well/100 µL	30,000 to 60,000 cells/well/25 µL
Long Incubation	Proliferation, tracking	10,000 to 20,000 cells/well/100 µL	5000 to 10,000 cells/well/ 50 µL

✓ Flow cytometry assays

Each cell line should be evaluated on the individual basis to determine the optimal cell density. For detaching adherent cells from the plate, 0.5 mM EDTA is recommended. Enzymatic reagents (e.g. trypsin,

Accutase) can be considered but need to be tested to make sure the receptor of interest on the cell surface is not affected.

- For adherent cells: Plate cells at 400,000 to 800,000 cells/mL in cell growth medium the day prior to use.
- For non-adherent cells:
  1. Centrifuge the cells and carefully discard the supernatant (i.e., the culture medium).
  2. Re-suspend the cell pellet in 500 µL – 1 mL cell growth medium or HHBS at 500,000 to 1,000,000 cells/mL .

✓ Other cell types

Below are some general guidelines for preparing/lysing plant, bacterial, mammalian or tissue cell samples. Note that each cell type should be evaluated on an individual basis to determine the optimal cell densities and conditions.

- For plant cells:
  1. Homogenize leaves with lysis buffer at 200 mg/mL.
  2. Centrifuge at 2500 rpm for 5-10 minutes.
  3. Use the supernatant for tests.
- Bacterial cells:
  1. Collect bacterial cells by centrifugation (10,000 g, 0 °C, 15 min).
  2. Add 1 mL of lysis buffer per 100 to 10 million cells and incubate the treated solution at room temperature for 15 minutes.
  3. Centrifuge at 2500 rpm for 5 minutes.
  4. Use the supernatant for tests.
- Mammalian cells:
  1. Remove medium from plate wells and add about 100 µL lysis buffer per 1 to 5 million cells (or 50-100 µL/well in a 96-well cell culture plate).
  2. Incubate the treated solution at room temperature for 15 minutes.
  3. Use the cell lysate directly or centrifuge at 1500 rpm for 5 minutes and then use the supernatant for tests.
- Tissue:
  1. Weigh ~20 mg tissue and wash with cold PBS.
  2. Homogenize with 400 µL of lysis buffer in a micro-centrifuge tube.
  3. Centrifuge at 2500 rpm for 5-10 minutes.
  4. Use the supernatant for the assay.

Assay Procedure

1. Add test compounds into the cells and incubate for a desired period of time (such as 24, 48 or 96 hours) in a 37°C, 5% CO_2 incubator. For blank wells (medium without the cells), add the same amount of compound buffer.

   Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density and

incubation time. We incubated CHO-K1 cells with 20 mM Glucose for glucose competition assay, and 100 µM Phloretin for GLUTs inhibition assay. See Data Analysis for details.

2. At the end of the treatment, centrifuge the plate for 5 minutes at 800 rpm with brake off prior to your experiment.
3. Aspirate the supernatant without disturbing cells.
4. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of 2-NBDG staining solution.
   Note: Optimal incubation time will need to be determined for each cell line and for each specific experiment. We incubated CHO-K1 cells at 37°C with 100 µM 2-NBDG (~34 µg/mL) for 20 minutes to show sufficient glucose uptake. See Data Analysis for details
5. At the end of the incubation, centrifuge the plate for 5 minutes at 800 rpm.
6. Remove 2-NBDG staining solution without disturbing cells.
7. For fluorescence microscope: Wash cells with Assay Buffer I (Component B) once. Keep cells in 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Assay Buffer II (Component C). Monitor the fluorescence signal using a fluorescence microscope with FITC filter.
8. For flow cytometer: Detach cells if required using EDTA and resuspend cells in 100 µL/sample of Assay Buffer I (Component B). Monitor the fluorescence signal using a flow cytometer with 530/30 nm filter (FITC channel).

✓ Summary

1. Prepare cells with your test compounds
2. Add 2-NBDG staining solution
3. Incubate cells at 37°C for 20 minutes
4. Remove 2-NBDG staining solution
5. Wash cells with Assay Buffer I
6. Analyze cells using fluorescence microscope or flow cytometer with 530/30 nm filter (FITC channel)

Important: Thaw all the components at room temperature before starting the experiment.

Data Analysis

Example Data Analysis and Figures

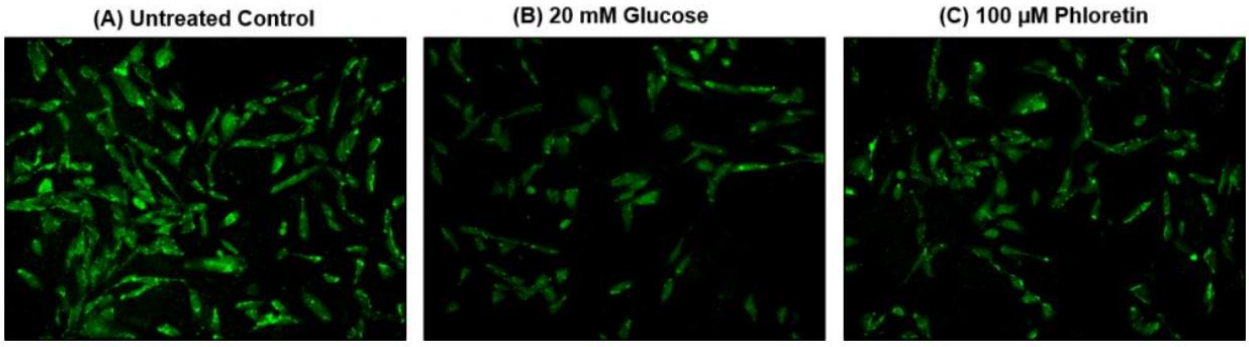

Figure 1. Fluorescence images of 2-NBDG uptake in CHO-K1 cells using the 2-NBDG Glucose Uptake Assay Kit. CHO-K1 cells at 40,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with 20 mM Glucose (B) or 100 µM Phloretin (C) at 37°C for 1 hour, then incubated with 100 µM 2-NBDG staining solution for 20 minutes. Untreated control cells were stained under the same conditions. The fluorescence signal was measured using a fluorescence microscope with FITC filter.