AAT Bioquest®

PRODUCT INFORMATION SHEET

Cell Meter™ 2-NBDG Glucose Uptake Assay Kit

Catalog number: 23500
Unit size: 200 Tests

Component	Storage	Amount
Component A: 2-NBDG (10 mg/mL)	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)
Component B: Assay Buffer I	Freeze (<-15 °C), Minimize light exposure	1 bottle (50 mL)
Component C: Assay Buffer II	Freeze (<-15 °C), Minimize light exposure	1 bottle (20 mL)

OVERVIEW

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. AAT Bioquest's Cell Meter™ 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). Cell Meter™ 2-NBDG Glucose Uptake Assay Kit is the most robust tool for monitoring glucose transporters.

AT A GLANCE

Protocol 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.

KEY PARAMETERS

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

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.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

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).

EXAMPLE DATA ANALYSIS AND FIGURES

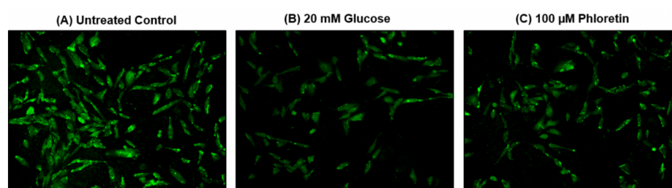

Figure 1. Fluorescence images of 2-NBDG uptake in CHO-K1 cells using Cell Meter™ 2-NBDG Glucose Uptake Assay Kit. CHO-K1 cells at 40,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with 20 mM Glucose (B) or 100 µM Phloretin (C) at 37°C for 1 hour, then

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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.

DISCLAIMER

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