Version 1 Last updated 30 August 2018

ab235976
2-NBDG Glucose Uptake Assay Kit

For the measurement of glucose uptake in cell cultures.

This product is for research use only and is not intended for diagnostic use.

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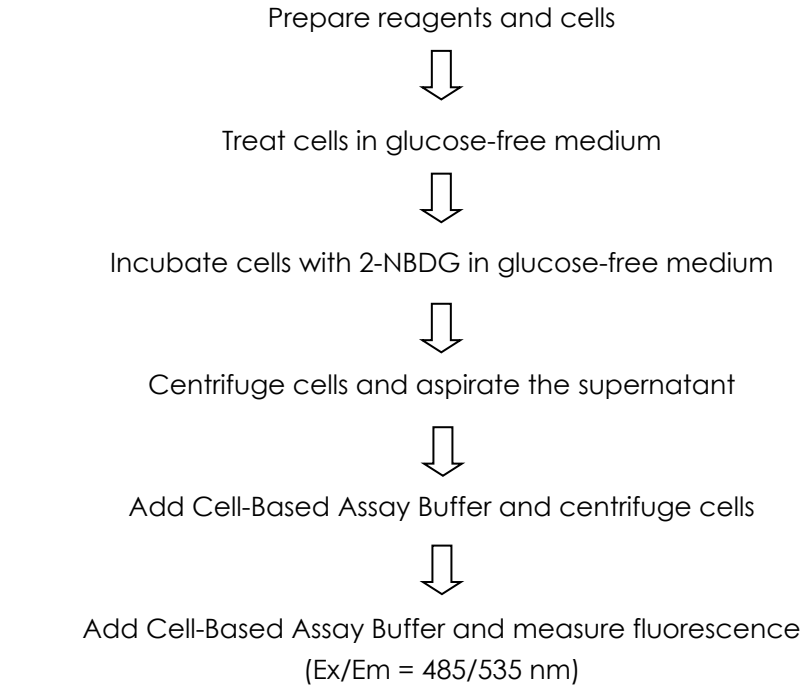
1. Overview

2-NBDG Glucose Uptake Assay Kit (ab235976) provides a convenient tool for studying modulators of cellular glucose uptake. The kit employs 2-NBDG, a fluorescently-labeled deoxyglucose analog, as a probe for the detection of glucose taken up by cultured cells. Researchers interested in cellular metabolism, such as cancer biologists, immunologists, physiologists, and others will find this kit a robust tool for measuring energy consumption in living cells.



2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Cell-Based Assay NBD Glucose	500 µL	-20°C	-20°C
Cell-Based Assay Apigenin	100 µL	-20°C	-20°C
Cell-Based Propidium Iodide Solution	250 µL	4°C	4°C
Cell-Based Assay Buffer Tablet	1 unit	RT	RT

3. Materials Required, Not Supplied

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

- Cell culture or staining plates: 96-well black with clear bottom for plate reader and microscopy, polypropylene v-bottom for flow cytometry.
- Cells which express GLUT1 glucose transporter (if Apigenin is to be used as inhibitor).
- A flow cytometer, microscope, or plate reader capable of detecting fluorescence at excitation and emission wavelengths of 485 nm and 535 nm, respectively.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

5.1 Cell-Based Assay Buffer Tablet

1. Dissolve the Cell-Based Assay Buffer Tablet in 100 mL of distilled water.

5.2 Cell-Based Assay NBD Glucose

1. This fluorescently-tagged glucose derivate is supplied as a solution in ethanol at 10 mg/mL (approximately 30 mM).
2. Dilute this fluorescent solution in 1/50-1/100 in the glucose-free culture medium used for your experiments.
3. The final concentration of 2-NBDG in the culture medium is 100-200 µg/mL.

ΔNote: The optimal concentration needed will depend on the cell lines and experimental designs.

5.3 Cell-Based Assay Apigenin

1. Apigenin is supplied at a concentration of 50 mM in DMSO. For inhibition of glucose uptake, it can be diluted in 1/500-1/1000 into gucose-free medium.

5.4 Cell-Based Propidium Iodide Solution

1. Ready to use as supplied.

6. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

ΔNote: The following protocol is designed for a 96-well plate. For fluorescence microscopy, use a black, clear bottom plate. For flow cytometry readouts, culture cells in any size plate and transfer to a 96-well v-bottom plate or FACS tubes for analysis. Adjust volumes accordingly for other sizes of plates.

6.1 Assay Procedure

1. Seed a 96-well plate with $1x10^4$ – $5x10^4$ cells/well in 100 µL culture medium. Grow cells overnight.
2. The next day, treat the cells with experimental compounds or vehicle control in 100 µL glucose-free culture medium.
3. Ten minutes before the end of the treatment, add 2-NBDG to a final concentration of 100-200 µg/mL in glucose-free medium.

ΔNote: The timing of incubation with 2-NBDG sufficient for showing differences in glucose uptake varies greatly with the cell line and experimental conditions, and may be as long as 16 hours. Optimal incubation time will need to be determined or each individual experiment.

4. At the end of the treatment, centrifuge the plate for 5 minutes at 400 x *g* at room temperature.
5. Aspirate the supernatant.
6. Add 200 µL of Cell-Based Assay Buffer to each well. Be careful not to disturb the cell layer.

ΔNote: For flow cytometric applications, Propidium Iodide can be added at this point to exclude dead cells, which take up this dye. Dilute the supplied Propidium Iodide Solution 1/1000 in Cell-Based Assay Buffer before adding 200 µL to the cells.

7. Centrifuge the plate for 5 minutes at 400 x *g* at room temperature.
8. Aspirate the supernatant.

9. Add 100 µL of Cell-Based Assay Buffer to each well. The cells are now ready for analysis and must be analyzed immediately. 2-NBDG taken up by the cells can be detected with fluorescent filters usually designed to detect fluorescein (excitation/emission = 485/535 nm). Propidium iodide fluoresces in dead cells only with Ex/Em = 488/650 nm, so gating on negative cells will exclude dead cells from analysis.

7. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

Problem	Reason	Solution
No glucose uptake in all treatments, including negative control	Cells are not healthy	Use only healthy cells
No significant difference in fluorescent staining intensity among treatments	Culture medium contains high level of glucose	Use culture medium which contains no glucose

8. Typical Data

Data provided **for demonstration purposes only**.

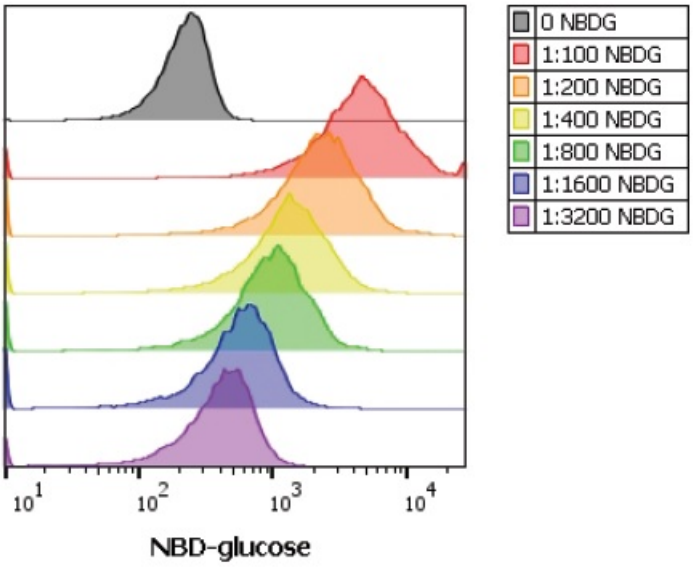


Figure 1. NBD-glucose is taken up by Jurkat (Human T cell leukemia cell line from peripheral blood) cells in a dose-dependent manner. Jurkat cells were equilibrated in glucose-free medium for two hours prior to being treated with the indicated dilution of NBD-glucose for 10 minutes at 37°C. Cells were washed and data were collected on a MACSQuant cytometer. NBD-glucose fluorescence within the live population is shown.

9. Notes

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