

**Glucose Uptake
Cell-Based Assay Kit**

Item No. 600470

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
600471	Cell-Based Assay NBD Glucose	1 vial/500 µl	-20°C
10009322	Cell-Based Assay Buffer Tablet	1 vial/1 tablet	Room Temperature
600472	Cell-Based Assay Apigenin	1 vial/100 µl	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



WARNING: This product is for laboratory research use only; not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A 6-, 12-, 24-, or 96-well plate
2. Cultured cells
3. A flow cytometer, microscope, or plate reader equipped with optics capable of detecting fluorescence at excitation and emission wavelengths of 485 nm and 535 nm, respectively
4. Adjustable pipettes and a repeat pipettor

INTRODUCTION

Background

Glucose is a ubiquitous energy source in most organisms, from bacteria to humans. Glucose metabolism, a process which converts glucose into energy in the form of adenosine triphosphate (ATP), is a primary source of energy and biomaterials for the maintenance of cell homeostasis.¹ In humans, glucose is absorbed by the intestines and then into the blood. Extra glucose is stored in the muscles and liver as glycogen which is hydrolyzed to glucose and released into the blood when needed. Blood glucose levels must be maintained within homeostatic levels to ensure optimal supply of glucose for normal cell function and survival. Glucose uptake in cells is achieved by the action of glucose transporters, which facilitate glucose movement down a concentration gradient, in contrast to energy-dependent uptake of glucose in the gut or kidney. The rate of glucose uptake in cells is dynamic and tightly regulated by hormones and/or growth factors including insulin. During growth and proliferation, there is an increase of glucose uptake to sustain energy demands while there is a decrease in glucose uptake when cells atrophy.²

Cancer cells exhibit alterations in metabolic pathways compared to normal cells. These include increased glucose uptake and metabolism by aerobic glycolysis in order to support a high rate of proliferation.^{3,4} Chemicals that block glucose uptake by cancer cells have been shown to have anti-cancer effects.⁵ In cancer drug discovery, there are renewed efforts to target cancer cell metabolism such as the glucose metabolic pathway. Use of a recently developed fluorescent glucose analog, 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl) amino]-D-glucose) (2-NBDG), has allowed drugs targeting glycolysis and glucose uptake to be developed.

About This Assay

Cayman's Glucose Uptake Cell-based Assay Kit 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. Apigenin, a flavonoid that has been reported to be an inhibitor of glucose transport, is included as a control.⁶

Reagent Preparation

1. Cell-Based Assay Buffer - (Item No. 10009322)

Dissolve the Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

2. Cell-Based Assay NBD Glucose - (Item No. 600471)

This fluorescently-tagged glucose derivative (2-NBDG) is supplied as a solution in ethanol at 10 mg/ml (approximately 30 mM). Dilute this fluorescent solution 1:50-1:100 in the glucose-free culture medium used for your experiments. The final concentration of 2-NBDG in the culture medium is 100-200 µg/ml. The optimal concentration needed will depend on the cell lines and experimental designs.

NOTE: Protect from light.

3. Cell-Based Assay Apigenin - (Item No. 600472)

Apigenin is supplied at a concentration of 50 mM in DMSO. It may be diluted directly into glucose- and serum-free media.

Fluorescence Microscopy

The following protocol is designed for a 96-well plate. Adjust volumes accordingly for other sizes of plates.

1. Seed a 96-well plate with 1×10^4 - 5×10^4 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 containing 150 µg/ml 2-NBDG (see page 6). Incubate the cells for 10 minutes, or for the period of time used in your typical experimental protocol. To use the included Apigenin as a control, dilute 1:1,000 in your culture medium.
3. At the end of the treatment, centrifuge the plate for five minutes at 400 x g at room temperature.
4. Aspirate the supernatant.
5. Add 200 µl of Cell-Based Assay Buffer to each well. Be careful not to disturb the cell layer.
6. Centrifuge the plate for five minutes at 400 x g at room temperature.
7. Aspirate the supernatant.
8. Add 100 µl of Cell-Based Assay Buffer to each well. The cells are now ready for analysis by fluorescent microscopy and must be analyzed immediately. 2-NBDG taken up by cells can be detected with fluorescent filters usually designed to detect fluorescein (excitation/emission = 485/535 nm).

Plate Reader

The following protocol is designed for a 96-well plate. Adjust volumes accordingly for other sizes of plates. A 96-well **black, clear bottom** culture plate should be used for this method. Optimal conditions will depend on the cell type.

1. Seed a 96-well black, clear bottom culture plate with 1×10^4 - 5×10^4 cells/well in 100 μ l culture medium. Grow cells overnight.
2. The next day, treat the cells with experimental compounds or vehicle control in 100 μ l glucose-free culture medium containing 150 μ g/ml 2-NBDG (see page 6). Incubate the cells for 10 minutes, or for the period of time used in your typical experimental protocol. To use the included Apigenin as a control, dilute 1:1,000 in your culture medium.

NOTE: Differences in the number of live cells can significantly affect results. Ensure that experimental compounds used do not significantly cause cell death.

3. At the end of the treatment, centrifuge the plate for five minutes at 400 x g at room temperature.
4. Aspirate the supernatant.
5. Add 200 μ l of Cell-Based Assay Buffer to each well. Be careful not to disturb the cell layer.
6. Centrifuge the plate for five minutes at 400 x g at room temperature.
7. Aspirate the supernatant.
8. Add 100 μ l of Cell-Based Assay Buffer to each well. The cells are now ready for analysis, and must be analyzed immediately. The amount of 2-NBDG taken up by cells can be measured at wavelengths usually designed to detect fluorescein (excitation/emission = 485/535 nm).

Flow Cytometry

1. Culture cells in 6-, 12-, or 24-well plates at a density of 5×10^5 cells/ml in a CO₂ incubator overnight at 37°C.
2. The next day, treat the cells with experimental compounds or vehicle control in 100 μ l glucose-free culture medium containing 150 μ g/ml 2-NBDG (see page 6). Incubate the cells for 10 minutes, or for the period of time used in your typical experimental protocol. To use the included Apigenin as a control, dilute 1:1,000 in your culture medium.
3. At the end of the treatment, harvest cells from each well into a plastic tube fitted for the flow cytometer.
4. Centrifuge the samples for five minutes at 400 x g at room temperature. Carefully aspirate the supernatant. Add 1 ml of Assay Buffer to each tube and vortex to ensure that all cells are suspended.
5. Centrifuge the cells at 400 x g for five minutes. Aspirate the supernatant.
6. Resuspend the cells in 0.5-1 ml of Assay Buffer. Mix well to ensure separation of individual cells.
7. Analyze the cells with a flow cytometer. The cells must be analyzed immediately. Cells taking up 2-NBDG display fluorescence with excitation and emission at 485 nm and 535 nm, respectively, and can be measured in the channel used to detect fluorescein.

Cell Staining

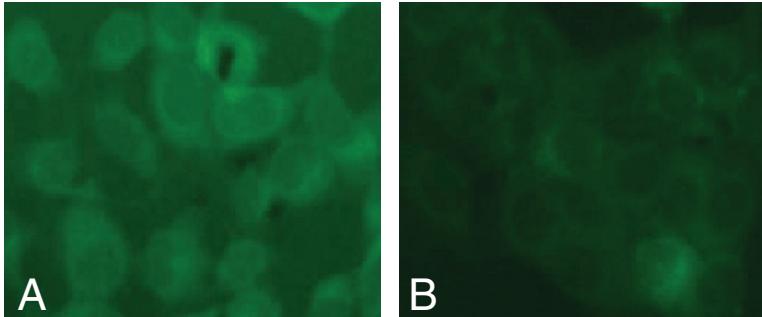


Figure 1. Apigenin decreases 2-NBDG uptake in MCF-7 cells as measured by fluorescent microscopy. MCF-7 cells were seeded at a density of 10,000 cells/well and incubated overnight in a cell culture incubator at 37°C. The next day, cells were treated with vehicle or 100 μM apigenin in culture medium containing no glucose, following immediately by addition of 150 μg/ml 2-NBDG to all wells. Cells were incubated in the same culture medium for 10 minutes. Cells were then washed with Assay Buffer once and examined under a microscope with filters used to detect fluorescein. *Panel A:* Cells grown in culture medium without glucose or FBS and treated with vehicle. There is abundant 2-NBDG uptake in these cells. *Panel B:* Cells grown in culture medium without glucose or FBS and treated with 100 μM Apigenin. There is a decrease in 2-NBDG uptake in these cells compared to those cells treated with vehicle, as evidenced by the significant drop in fluorescence intensity.

Plate Reader

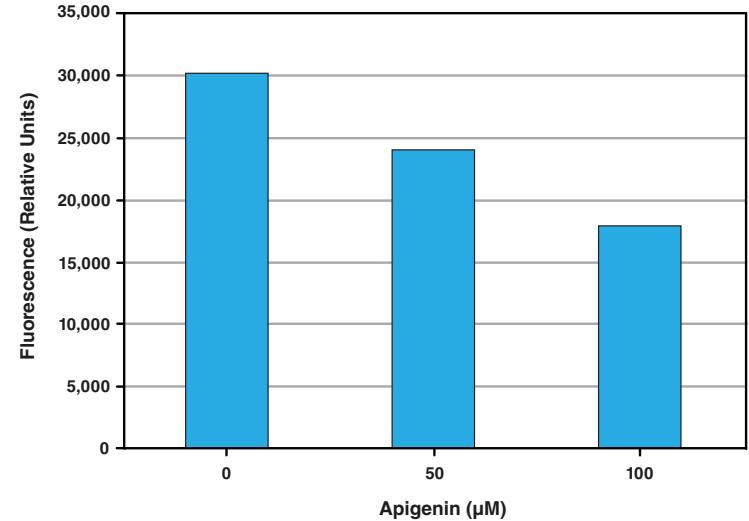


Figure 2. Measurement of 2-NBDG uptake in LS-180 cells grown in glucose- and serum-free medium and treated with various doses of Apigenin. LS-180 cells were seeded at a density of 10,000 cells/well and incubated overnight in a cell culture incubator at 37°C. The next day, cells were treated with vehicle or different concentrations of Apigenin in glucose- and serum-free culture medium. 2-NBDG (150 μg/ml) was added to all wells at the same time as treatment. Cells were then incubated in the same culture medium overnight. At the end of the experiment, the degree of 2-NBDG uptake was analyzed using a plate reader. Apigenin causes a decrease in 2-NBDG uptake in LS-180 cells in a dose dependent manner, as indicated by the change in fluorescence intensity.

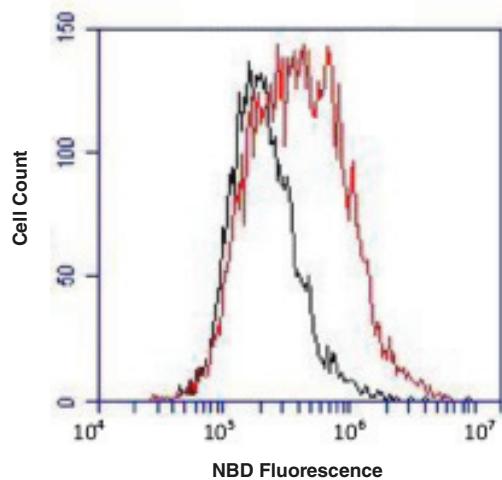


Figure 3. Apigenin decreases glucose uptake in Jurkat cells. Jurkat cells were plated at a density of 1×10^6 cells/well in a 6-well plate and cultured in glucose free RPMI medium containing vehicle or 100 μ M Apigenin. After four hours of treatment, 150 μ g/ml 2-NBDG was added to each well of cells. After 10 minutes of incubation, the amount of 2-NBDG taken up by cells from different treatments was analyzed with an Accuri™ C6 Flow Cytometer. Apigenin at 100 μ M (black) caused a significant decrease in glucose uptake, as evidenced by the significant shift of the cell population to lower fluorescence.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
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

References

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NOTES

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