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	RT
600472	Cell-Based Assay Apigenin	1 vial/100 µl	-20°C
10011234	Propidium Iodide Solution	1 vial/250 µl	4°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3640

Email: techserv@caymanchem.com

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 2, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

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

INTRODUCTION

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 mediated by GLUT1, is included as a control.[1] 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.

PRE-ASSAY PREPARATION

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. For inhibition of glucose uptake, it can be diluted 1:500-1:1,000 into glucose-free medium.

ASSAY PROTOCOL

Performing the Assay

The following protocol is designed for a 96-well plate. For fluorescence microscopy, use a black, clear bottom plate. For flow cytometric 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.

1. Seed a 96-well plate with 1 x 10^4 - 5 x 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. Ten minutes before the end of the treatment, add 2-NBDG to a final concentration of 100-200 µg/ml in glucose-free medium. 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 for each individual experiment.
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. *Note: 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 (Item No. 10011234) 1:1,000 in Cell-Based Assay Buffer before adding 200 µl to the cells.*
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 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). Propidium iodide fluoresces in dead cells only with ex/em 488/650 nm, so gating on negative cells will exclude dead cells from analysis.

PERFORMANCE CHARACTERISTICS

Flow Cytometry

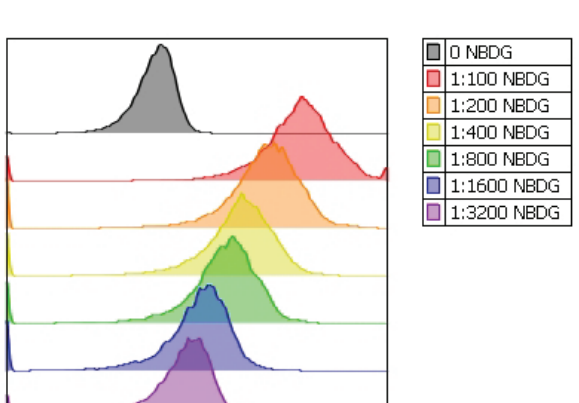

Figure 1: NBD-glucose is taken up by Jurkat cells in a dose-dependent manner. Jurkat cells were equilibrated in glucose-free medium for two hours prior to being treated with the indicated dilutions of NBD-glucose for 10 minutes at 37°C. Cells were washed and data were collected on a MACSQuant cytometer. NBD-glucose fluorescence within the live population is shown.

RESOURCES

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

Reference

1. Park, J.B. Flavonoids are potential inhibitors of glucose uptake in U937 cells. *Biochem. Biophys. Res. Commun.* **260(2)**, 568-574 (1999).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.