2-NBDG Glucose Uptake Assay Kit

Introduction

Glucose metabolism is a process that converts ingested glucose into energy, which is the main source of energy supply in living organisms. Studies have shown that glucose metabolism is closely related to many diseases such as cancer, diabetes and obesity, so it is important to monitor glucose uptake.

The 2-NBDG Glucose Uptake Assay Kit is a kit for the detection of glucose uptake by the fluorescent probe 2-NBDG. 2-NBDG is a fluorescently labeled glucose analogue that is transported into cells by glucose transporter (GLUT) like glucose. Once 2-NBDG is uptaken in cells, it is phosphorylated at the C-6 position to produce 2-NBDG-6-phosphate, which is well retained in cells. Compared to other glucose trackers (2-DG or FDG), 2-NBDG allows in situ detection of glucose uptake at the single-cell level.

This kit provides a sensitive, rapid and non-radioactive assay to detect glucose uptake. At the same time, the kit provides phloretin, a GLUT1 inhibitor that can significantly inhibit glucose uptake, as a positive control. For 96-well plates, using 100 μL of working solution per well, the kit can be used at least 500 times.

Components and Storage

Components	K2212-500 T
2-NBDG	500 μL
PI (1000x)	250 μL
Phloretin (100x)	100 μL
5x Assay Buffer	20 mL
Store the kit at -20°C, stable for 1 year. 2-NBDG and PI (1000x) should be stored away from light.	

Protocol

1. **Reagent preparation:**

   1) **1x Assay Buffer preparation:** Dilute appropriate 5x Assay Buffer in sterile water to make 1x Assay Buffer.

   2) **2-NBDG working solution preparation:** Dilute appropriate 2-NBDG in the glucose-free medium in a ratio of 1:100-1:200 to make a 2-NBDG working solution. Prepare fresh 2-NBDG working solution every time. The 2-NBDG staining solution is stable for 1 hour at room temperature.

*Note: The optimal concentration of 2-NBDG working solution varies depending on the cell types.

3) **PI working solution preparation:** Dilute appropriate PI (1000x) in 1x Assay Buffer in a ratio of 1:1000 to make the PI working solution.

2. **Cell culture:** Seed cells in Cells in suitable plates, petri dishes or flasks, and the cell should be 70-90% confluent before the experiment.

3. **Drug treatment:** Remove the culture medium, the interested drug can be added at a dilution in the glucose-free medium for a desired period. For blank wells (medium without the cells), add the same amount of drug.

*Note: Because glucose is the largest source of interference for this kit, it is necessary to dilute the drug with the glucose-free medium.

4. **Positive control (optional):** Dilute Phloretin (100x) in the glucose-free medium in a ratio of 1:100 to make the Phloretin working solution. For the positive control, incubate with Phloretin working solution at 37°C for a desired period.

*Note: The optimal concentration and incubation time of Phloretin working solution varies depending on the cell types. When dilution at 1:100, treatment for 1 h is enough for most cells.

5. **2-NBDG staining:** Centrifuge the plate for 5 minutes at 800 rpm at room temperature to remove the drug. Incubate with 2-NBDG working solution at 37°C for a desired period. For 96-well pates, add 100 µL of 2-NBDG working solution to each well. Other plate sizes can be used by scaling as necessary, but make sure that the working solution can fully cover the cells. After incubation, centrifuge the plate for 5 minutes at 800 rpm at room temperature to remove the 2-NBDG working solution.

*Note: The optimal incubation time of 2-NBDG working solution varies depending on the cell types.

6. **PI staining (optional):** Wash cells 1-2 times with 1x Assay Buffer, and incubate with PI working solution at room temperature for 3-5 min in the dark. After incubation, centrifuge the plate for 5 minutes at 800 rpm at room temperature to remove the PI working solution.

*Note: This step is required for flow cytometry assays to exclude dead cells, which can be optionally performed for fluorescence microscopy.

7. **Washing:** Wash cells 1-2 times with 1x Assay Buffer. Add an appropriate amount of 1x Assay Buffer and detect immediately.

8. **Detection:** Monitor the fluorescence signal of 2-NBDG with Ex/Em=485/535 nm. PI fluoresces in dead cells with Ex/Em=488/650 nm, so gating on negative cells needs to exclude dead cells for flow cytometry detection.

*Note:

a) The detection needs to be performed as soon as possible after staining, otherwise it may affect the result.

b) When using a fluorescence microscope, it is recommended to minimize the intensity of the excitation light and shorten the exposure time to minimize fluorescence quenching.

c) For adherent cells, detach cells using EDTA and resuspend cells in an appropriate amount of 1x Assay Buffer before flow

cytometry detection.

Note

1. The detection needs to be performed as soon as possible after staining, otherwise it may affect the result.
2. 2-NBDG is easy to quench and needs to be protected from light when storing and using. At the same time, it is recommended to aliquot appropriately to avoid repeated freezing and thawing.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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