2-NBDG Glucose Uptake Cell-Based Kit

Cat. No: E-CK-A441 **Size: 50 Assays/200 Assays**

Cat.	Products	50 Assays	200 Assays	Storage
E-CK-A441A	2-NBDG Reagent (10 mM)	500 µL	500 µL×4	-20°C, shading light
E-CK-A441B	2-NBDG Uptake Buffer	10 mL	10 mL×4	-20°C, shading light
Manual			One Copy	

Storage

2-NBDG Reagent (10 mM) and 2-NBDG Uptake Buffer can be stored at -20°C with shading light for 1 year.

Detection Principle

2-NBDG Reagent is a green fluorescent labeled (Ex/Em=475nm/550nm) deoxyglucose analog, synthesized by the reaction of D-glucosamine with NBD-CI, which has the same transporters (GLUTs) and similar transport kinetics as D-glucose. 2-NBDG Reagent is phosphorylated by hexokinase at the C-6 position after cellular uptake, which causes the cells to emit green fluorescence. The fluorescence intensity reflects the glucose uptake of the cells. 2-NBDG Reagent can be used as a probe to determine the glucose uptake ability of living cells, which is one of the indicators for determining the viability of the cells, and it can also be used in the evaluation and screening of drugs for glucose metabolism diseases.

Detection Sample Types

☑ Adherent Cells

☑ Suspension Cells

Materials Not Supplied

1) **Reagents**

PBS, cell culture medium, fetal bovine serum

2) **Instruments**

Flow cytometer, inverted fluorescence microscope, biosafety cabinet, CO_2 Incubator

3) **Materials**

Petri dishes, 24-well plates, cell crawlers, microscope slides

Experimental Protocol

➢ For fluorescence microscopy

a) Carefully aspirate and discard the medium from the adherent cells, add 1 mL of PBS buffer per well to infiltrate and wash the cells for 3 min, and remove the PBS buffer.

b) **Preparation of 2-NBDG staining working solution (500 μM):** Bring the 2-NBDG Reagent (10 mM) and 2-NBDG Uptake Buffer to room temperature in advance, vortex the reagents and mix fully, then centrifuge before use. Then prepare 2-NBDG staining working solution (500 μM) according to the number of samples. Please refer to the table below (100 μL Working Solution per well for 96-well plates or 200 μL per well for 24-well plates).

Component	2-NBDG staining working solution (500 μM)			
2-NBDG Reagent (10 mM)	10 μL	25 μL	50 μL	100 μL
2-NBDG Uptake Buffer	200 μL	500 μL	1000 μL	2000 μL

Note: A negative control is recommended for each experiment. The negative control is the cells without treatment that resuspended in 2-NBDG Uptake Buffer and without 2-NBDG Reagent.

c) Add 2-NBDG staining solution (500 μM) and incubate for 60~120 min at 37℃ incubator with shading light.

Note: The glucose uptake capacity of different cells varies, and it is recommended to conduct preliminary experiments to determine the optimal incubation time.

d) Carefully aspirate the 2-NBDG staining working solution, add 1 mL of PBS buffer to each well, wash the cells for 3~5 min, remove the PBS buffer, add 500 μL of PBS buffer to infiltrate the cells.

e) Observed and photographed directly under an inverted fluorescence microscope. (2-NBDG probe is green fluorescent, Ex/Em= 475nm/550nm).

f) If the adherent cells are adhered to the glass crawler in advance, the cell crawler can also be removed after staining, placed on a slide, and then observed and photographed using an orthogonal fluorescence microscope.

Note: Keep the sample moist during the experiment to prevent the failure of the experiment caused by dry slides.

g) If the cells are in suspension, collect the cell pellet, add 500 μL of 2-NBDG Reagent Staining Solution (500 μM) to $1\sim5\times10^5$ cells to resuspend the cell pellet, incubate the cells at 37℃ with shading light for 60 min~120 min, add 1 mL of PBS buffer to wash the cells, centrifuge at 300g for 5 minand discard the supernatant, then take 10-20 μL of PBS buffer to resuspend the cell pellet, drop

on the slide and gently cover the coverslip to observe and take pictures under the microscope.

Note: It is recommended to use freshly prepared the staining working solution be dispensed and use out in the same day.

➢ **For flow cytometry**

a) Collect the cells and centrifuge at 300g for 3 min at room temperature, discard the supernatant, resuspend the cell pellet with 5 mL of PBS buffer, then centrifuge at 300g for 3 min at room temperature and keep the cell pellet.

b) **Preparation of 2-NBDG staining working solution (100 μM):** Compared with fluorescence microscopy, flow cytometry is more sensitive, and the optimal working solution concentration for 2-NBDG staining is 100 μM. Bring the 2-NBDG Reagent (10 mM) and 2-NBDG Uptake Buffer to room temperature in advance, vortex the reagents and mix fully, then centrifuge before use. Then prepare 2-NBDG staining working solution (100 μM) according to the number of samples. Please refer to the table below (200 μL Working Solution per 1~5×10^5 cells).

Component	2-NBDG staining working solution (100 μM)			
2-NBDG Reagent (10 mM)	2 μL	5 μL	10 μL	20 μL
2-NBDG Uptake Buffer	200 μL	500 μL	1000 μL	2000 μL

Note: A negative control is recommended for each experiment. The negative control is the cells without treatment that resuspended in 2-NBDG Uptake Buffer and without 2-NBDG Reagent.

c) Take 1~5 × 10^5 cells per group, add 200 μL 2-NBDG staining working solution (100 μM) to resuspend the cell pellet, gently mix fully, incubate at 37℃ in the incubator for 60 min with shading light.

d) Add 1 mL of PBS buffer to each group, gently mix fully, centrifuge at 300g for 3 min at room temperature, and discard the supernatant.

e) Resuspend the cells with 1 mL of PBS buffer, gently mix fully, centrifuge at 300g for 3 min at room temperature, and discard the supernatant.

f) Resuspend the cells with 100~200 μL of PBS buffer and detect with flow cytometer in FITC channel.

Note:

a）Detection of 2-NBDG Reagent by flow cytometry , FITC channel can be used.

b）If the glucose uptake capacity of the cells is weak, the incubation time can be extended appropriately.

Typical Results

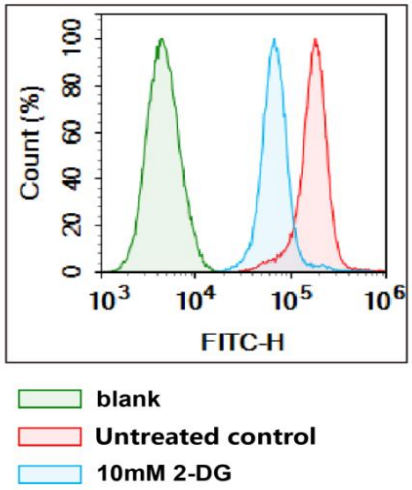

Figure 1. Jurkat cells were cultured with 10 mM 2-DG or without 2-DG (Untreated control) for 60 min, washed with PBS and incubated with 2-NBDG Glucose Uptake Cell-Based Kit for 60 min, and glucose uptake capacity of the cells was detected by flow cytometry, the results showed that 2-DG can inhibit glucose uptake in Jurkat cells.

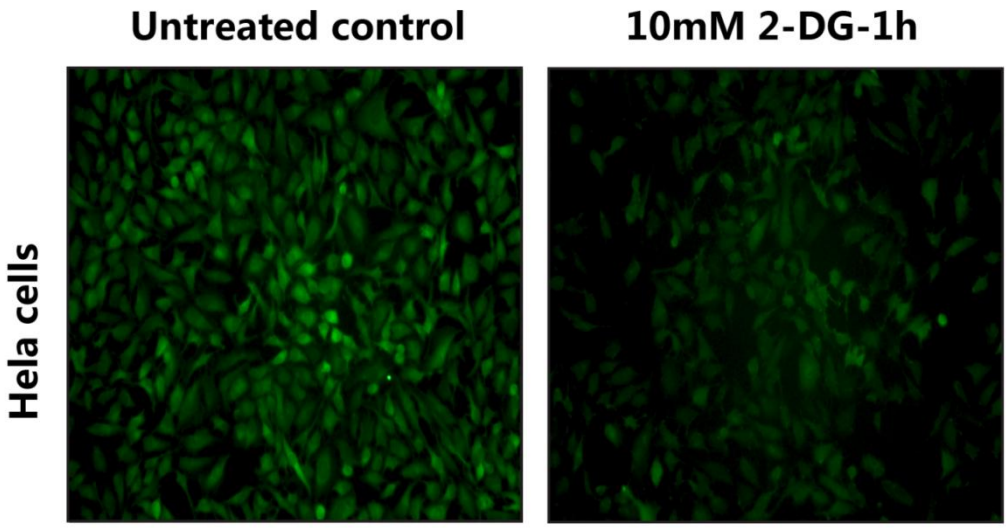

Figure 2. Hela cells were cultured with (left) or without 10 mM 2-DG (right) for 60 min. washed with PBS and incubated with 2-NBDG Glucose Uptake Cell-Based Kit for 60 min, and glucose uptake capacity was detected by fluorescence microscopy, the results showed that 2-DG can inhibit glucose uptake in Hela cells.

Cautions

1. This product is for research use only.
2. For your safety and health, please wear laboratory overalls and disposable gloves for operation, and follow the laboratory reagent operating procedures.
3. This product has fluorescence quenching problem, please store at -20℃ away from light; If it is not used for a long time, please store it at -80 ℃ after proper packaging. In order to ensure the effect of use, it is recommended to use within 6 months.