Glucose Uptake Assay Kit-Green (20 tests) *Technical Manual*

General Information

Nutrient metabolism is necessary for energy production in cells and regulates various cellular functions, including gene expression. Glucose is one of the key substrates for the generation of ATP and to sustain cellular homeostasis. Thus, glucose metabolism has been the subject of intense investigations. In cancer research, tumor cells enhance glucose uptake and consumption for their growth and proliferation. Therefore, elevated glucose uptake is a marker of tumors, and glucose transporters are important targets in cancer treatment.

One common method for evaluating the glucose uptake ability of cells uses radioisotope-labeled glucose. Although this method has been used for many years, it requires special handling facilities and disposal of radioactive materials. The enzyme cycling method using 2-deoxy-D-glucose, which enables colorimetric and fluorometric plate assays, cannot be applied to cell imaging and flow cytometry. Recently, 2-NBDG, a fluorescently labeled glucose analog, has been used widely to detect cellular glucose uptake by fluorescence imaging and flow cytometry[1)]. However, the sensitivity of this method is poor because of the low fluorescence intensity of 2-NBDG.

To resolve these limitations, a novel fluorescent probe, Glucose Uptake Probe-Green, was developed. This probe emits strong green fluorescence (λ_{ex} = 507 nm, λ_{em} = 518 nm), allowing highly sensitive detection of cellular glucose uptake by fluorescence imaging, flow cytometry or microplate assay. The WI Solution in this kit enhances cellular retention of the probe to give more reliable data.

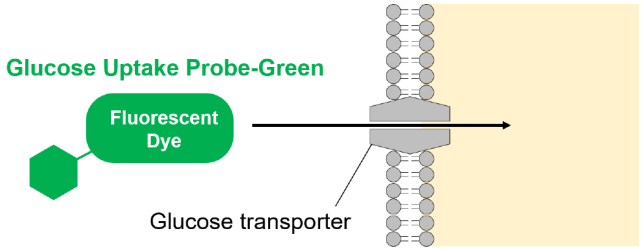


Figure 1. Principle of Glucose Uptake Assay Kit-Green

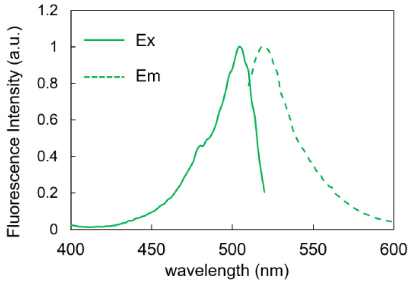


Figure 2. Excitation and emission spectra of Glucose Uptake Probe-Green

Kit Contents

Glucose Uptake Probe-Green	×1
WI Solution (50x)	1 ml ×1

Storage Conditions

Store in a cool (0–5°C), dark and dry place.

Required Equipment and Materials

-Micropipette
-Dimethylsulfoxide (DMSO)
-HBSS (Hanks' Balanced Salt Solution)
-Medium (glucose-free, serum-free)
-Microtubes (1.5 ml)

Precautions

Tap the tube containing the probe before opening and open it with care. The content may have moved from the bottom of the tube during shipping.

Preparation of Solutions

1. Preparation of Probe stock solution

Add 8 µl of DMSO to the Glucose Uptake Probe-Green tube and dissolve by pipetting and vortex mixing.
Note: Store the Probe stock solution at -20°C, protect from light. Probe stock solution is stable at -20°C for 1 month.

2. Preparation of Probe solution

Dilute Probe stock solution 500-fold with glucose- and serum-free medium.

	Adherent Cells				Suspension Cells
Vessel (amount)	6-well (1.5 ml/well)	24-well (0.3 ml/well)	96-well (0.15 ml/well)	35-mm dish (1.5 ml/well)	1.5 ml microtube (0.5 ml/tube)
medium	1500 µl	300 µl	150 µl	1500 µl	500 µl
Probe stock solution	3 µl	0.6 µl	0.3 µl	3 µl	1 µl

3. Preparation of WI Solution (1x)

Dilute WI Solution (50x) 50-fold with HBSS.

	Adherent Cells				Suspension Cells
Vessel (amount)	6-well (1.5 ml/well)	24-well (0.3 ml/well)	96-well (0.15 ml/well)	35-mm dish (1.5 ml/well)	1.5 ml microtube (0.5 ml/tube)
HBSS	6000 µl	1200 µl	600 µl	6000 µl	500 µl
WI Solution (50x)	120 µl	24 µl	12 µl	120 µl	10 µl

Note: The indicated volumes are sufficient for three washes and imaging.

General Protocol

1. Seed cells on a dish or a microplate. Culture the cells at 37°C overnight in a 5% CO_2 incubator.
2. Remove the culture medium and wash the cells with pre-warmed glucose- and serum-free medium[*1] twice.
3. Add pre-warmed glucose- and serum-free medium[*1] and incubate at 37°C for 15 minutes in a 5% CO_2 incubator.
4. Remove the supernatant and add pre-warmed Probe solution[*1].
5. Incubate at 37°C for 15 minutes in a 5% CO_2 incubator.
6. Remove the supernatant and wash the cells with ice-cold WI Solution (1x) [*2] twice.
7. Remove the supernatant, add ice-cold WI Solution (1x) [*2], and incubate at room temperature for 5 minutes.[*3]
8. Remove the supernatant and add ice-cold WI Solution (1x) [*2].
9. Observe the cells under a fluorescence microscope.[*4, 5]

*1 Pre-warm the culture medium and Probe solution in an incubater (37°C). Glucose uptake into the cells may be affected by the temperature of culture medium and Probe solution.
*2 Please use the ice-cold WI Solution (1x) to prevent leakage of the probe from the cells.
*3 This step is necessary to avoid a high background.
*4 This kit is also applicable for flow cytometry. When using adherent cells, prepare a cell suspension using trypsin or a cell scraper. Glucose Uptake Assay Kit-Green and Glucose Uptake Assay Kit-Red are also applicable for microplate assay.
*5 If the background fluorescence is high, wash the cells with ice-cold WI Solution again.

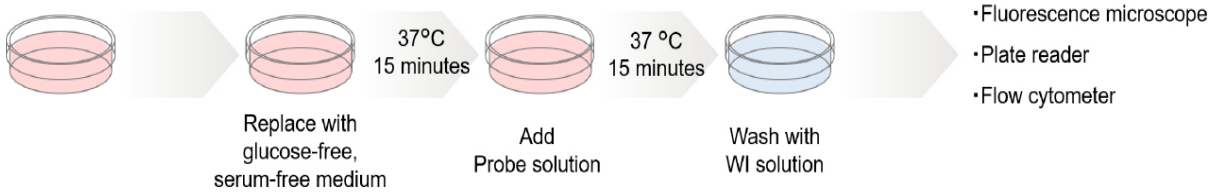


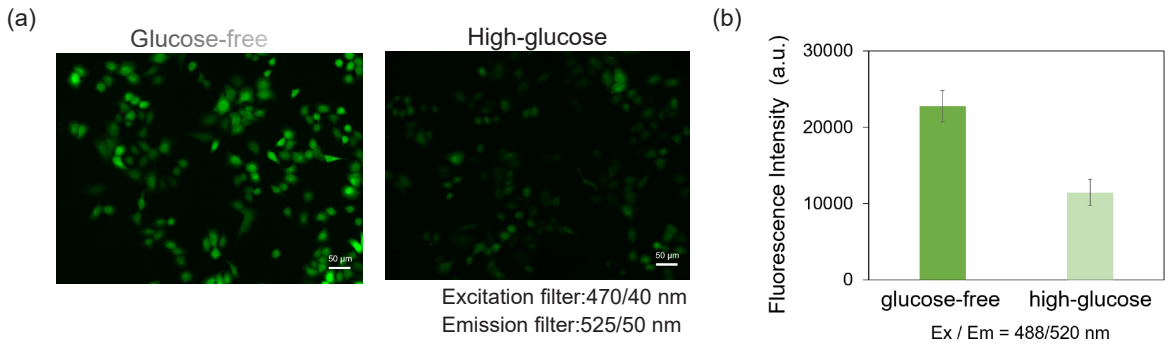
Figure 3. Protocol of Glucose Uptake Assay Kit-Green

Experimental Examples

Competitive inhibition of the probe uptake by D-glucose (A549 cells)

① Fluorescence microscopy and microplate reading

1. A549 cells (1.5×10^4 cells/well, 150 µl) in MEM (10% FBS) were seeded in a 96-well microplate (ib89626 : ibidi GmbH) and cultured at 37°C overnight in a 5% CO_2 incubator.
2. After removing the supernatant, the cells were washed twice with 150 µl of DMEM (glucose- and serum-free, 37°C).
3. DMEM (150 µl, glucose- and serum-free, 37°C) was added and the cells were incubated at 37°C for 15 minutes in a 5% CO_2 incubator.
4. After removing the supernatant, 150 µl of Probe solution in DMEM (glucose- and serum-free, 37°C) or DMEM (high-glucose, serum-free, 37°C) were added and the cells were incubated at 37°C for 15 minutes in a 5% CO_2 incubator.
5. After removing the supernatant, the cells were washed three times with 150 µl of WI Solution (1x, 4°C).
6. The cells were observed under a fluorescence microscope (GFP filter; BZ-X700, Keyence Corporation) and the fluorescence intensity was measured with a microplate reader (Ex / Em = 488/520 nm; Infinite m200 PRO, Tecan Trading AG, bottom reading).

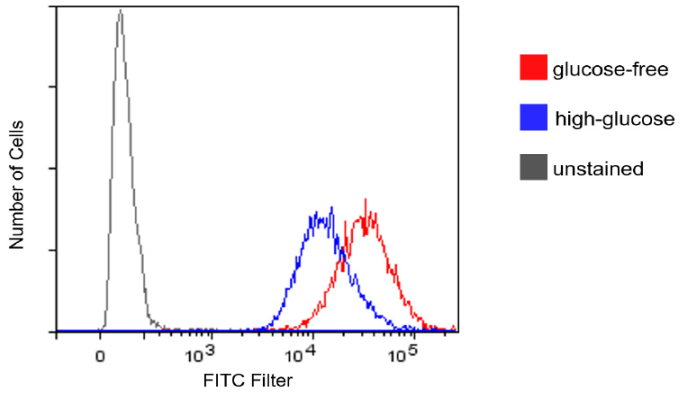


Glucose-free: A549 cells incubated with the probes in DMEM (glucose- and serum-free)
High-glucose: A549 cells incubated with the probes in DMEM (high-glucose, serum-free)

Figure 4. Competitive inhibition of the probe uptake by D-glucose (A549 cells)
(a) Fluorescence imaging; (b) plate reading.

② Flow cytometry

1. A549 cells (2.0×10^5 cells/well, 1.5 ml) in MEM (10% FBS) were seeded in a 6-well microplate (3810-006, AGC Techno Glass Co., Ltd.) and cultured at 37°C overnight in a 5% CO_2 incubator.
2. After removing the supernatant, the cells were washed twice with 1.5 ml of DMEM (glucose- and serum-free, 37°C).
3. DMEM (1.5 ml, glucose- and serum-free, 37°C) was added and the cells were incubated at 37°C for 15 minutes in a 5% CO_2 incubator.
4. After removing the supernatant, 1.5 ml of Probe solution in DMEM (glucose- and serum-free, 37°C) or DMEM (high-glucose, serum-free, 37°C) were added and the cells were incubated at 37°C for 15 minutes in a 5% CO_2 incubator.
5. After removing the supernatant, the cells were washed three times with 1.5 ml of WI Solution (1x, 4°C).
6. WI Solution (1x, 1.5 ml, 4°C) was added and the cells were harvested using a cell scraper.
   Note: The cells were kept in an ice bath until the measurement.
7. The cells were measured using a flow cytometer (filter set: FITC; LSR-Fortessa X-20, Becton, Dickinson and Company).



Ex/Em = 488/530 nm

Glucose-free: A549 cells incubated with the probes in DMEM (glucose- and serum-free)
High-glucose: A549 cells incubated with the probes in DMEM (high-glucose, serum-free)

Figure 5. Competitive inhibition of the probe uptake by D-glucose (A549 cells) measured by flow cytometry

Experimental Examples

Inhibition of the probe uptake by Cytochalasin B (HepG2 cells)

1. HepG2 cells (4.5×10^4 cells/well, 150 µl) in MEM (10% FBS) were seeded in a 96-well microplate (ib89626 : ibidi GmbH) and cultured at 37°C overnight in a 5% CO_2 incubator.
2. After removing the supernatant, 150 µl of MEM (10% FBS) containing 0 or 5 µmol/l Cytochalasin B were added, and the cells were incubated at 37°C overnight in a 5% CO_2 incubator.
3. After removing the supernatant, the cells were washed twice with 150 µl of DMEM (glucose- and serum-free, 37°C).
4. DMEM (150 µl, glucose-free, serum-free, 37°C) was added, and the cells were incubated at 37°C for 15 minutes in a 5% CO_2 incubator.
5. After removing the supernatant, 150 µl of Probe solution in DMEM (glucose- and serum-free, 37°C) were added and the cells were incubated at 37°C for 15 minutes in a 5% CO_2 incubator.
6. After removing the supernatant, the cells were washed three times with 150 µl of WI Solution (1x, 4°C).
7. The cells were observed under a fluorescence microscope (GFP filter; BZ-X700, Keyence Corporation) and the fluorescence intensity was measured with a microplate reader (Ex / Em = 488/520 nm; Infinite m200 PRO, Tecan Trading AG, top reading).

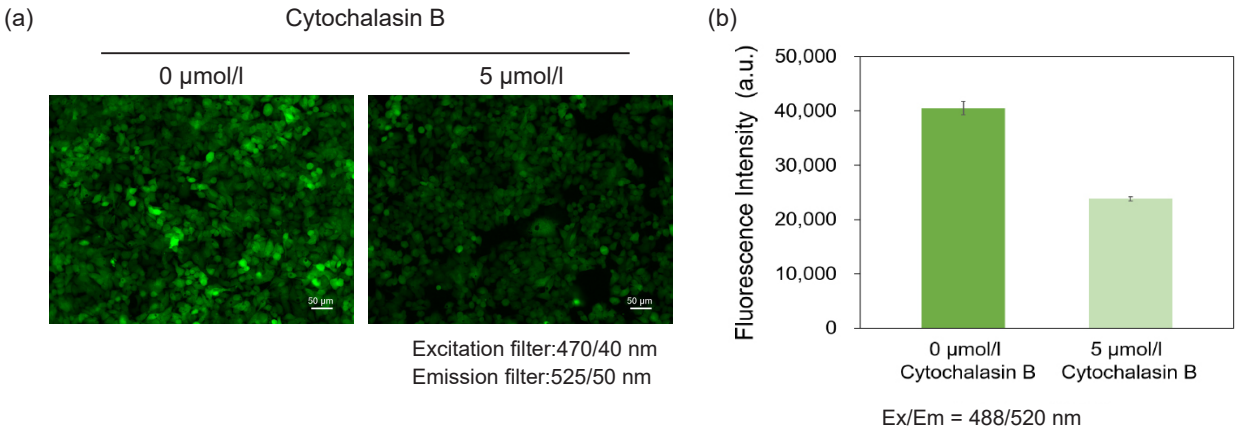


Figure 6. Inhibition of the probe uptake by Cytochalasin B (HepG2 cells)
(a) Fluorescence imaging; (b) plate reading.

Experimental Example

Measurement of increased uptake glucose level following adipocyte differentiation

-Adipocyte differentiation-

1. 3T3-L1 cells (1.5×10^4 cells/well, 150 µl) in DMEM (10% FBS) were seeded in a 96-well microplate (ib89626: ibidi GmbH) and cultured at 37°C overnight in a 5% CO_2 incubator.
2. The cells were cultured until the confluence. The culture medium was exchanged every 2 days interval.
   Note: Cells prepared without the following steps 3 and 4 were used as preadipocytes.
3. After removing the supernatant, 150 µl of DMEM (5.5 mmol/l glucose, 10% FBS) containing 1 µmol/l dexamethasone and 0.5 mmol/l 3-isobutyl-1-methylxanthine were added and the cells were cultured at 37°C for 2 days in a 5% CO_2 incubator.
4. After removing the supernatant, 150 µl of DMEM (5.5 mmol/l glucose, 10% FBS) containing 1.72 µmol/l insulin was added and the cells were cultured at 37°C for 2 days in a 5% CO_2 incubator.
5. After removing the supernatant, 150 µl of DMEM (5.5 mmol/l glucose, 10% FBS) were added and the cells were cultured at 37°C for 6 days in a 5% CO_2 incubator. The culture medium was exchanged every 2 days interval during the cell culture.

-Uptake of the probe by preadipocytes and adipocytes-

1. After removing the supernatant, the cells were washed twice with 150 µl of DMEM (glucose- and serum-free, 37°C).
2. DMEM (glucose- and serum-free, 37°C) was added and the cells were incubated at 37°C for 15 minutes in a 5% CO_2 incubator.
3. After removing the supernatant, 150 µl of Probe solution in DMEM (glucose- and serum-free, 37°C) was added and the cells were incubated at 37°C for 15 minutes in a 5% CO_2 incubator.
4. After removing the supernatant, the cells were washed three times with 150 µl of WI Solution (1x, 4°C).
5. The cells were observed under a fluorescence microscope (GFP filter; BZ-X700, Keyence Corporation).

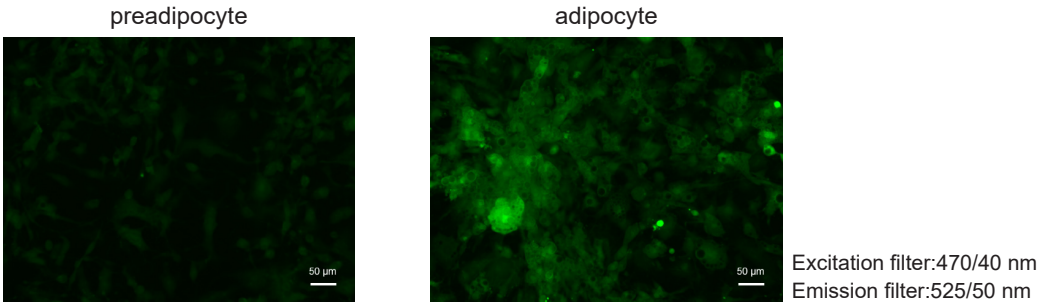


Figure 7. Enhancement of glucose uptake by differentiation

Reference

1) Yoshioka, K.; Takahashi, H.; Homma, T.; Saito, M.; Oh, B. K.; Nemoto, Y.; Matsuoka, H. "A novel fluorescent derivative of glucose applicable to the assessment of glucose uptake activity of *Escherichia coli. Biochim" Biophys. Acta,* **1996**, *1289*, 5-9.

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