Glucose Uptake Assay Kit-Blue
Glucose Uptake Assay Kit-Green
Glucose Uptake Assay Kit-Red

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 sustaining 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 standard 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, novel fluorescent probes, Glucose Uptake Probes, were developed. These probes emit strong fluorescence, allowing highly sensitive detection of cellular glucose uptake by fluorescence imaging, flow cytometry or microplate assay. The WI Solution in these kits enhances cellular retention of the probe to give more reliable data.

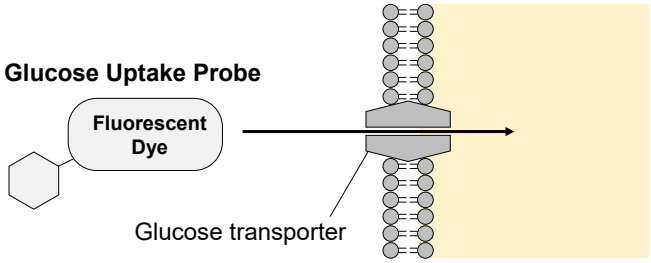


Figure 1. Principle of Glucose Uptake Assay Kit

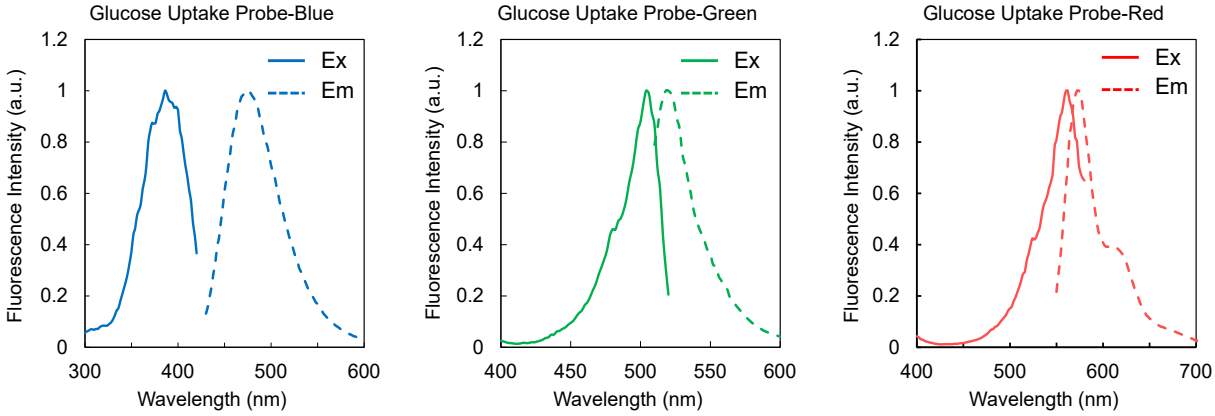


Figure 2. Excitation and emission spectra of Glucose Uptake Probes

Kit Contents

UP01	Glucose Uptake Probe-Blue	×1
	WI Solution (50x)	5 ml ×1
UP02	Glucose Uptake Probe-Green	×1
	WI Solution (50x)	5 ml ×1
UP03	Glucose Uptake Probe-Red	×1
	WI Solution (50x)	5 ml ×1

Storage Conditions

Store at 0–5°C

Required Equipment and Materials

-Micropipette
-Dimethylsulfoxide (DMSO)
-HBSS (Hanks' Balanced Salt Solution)
-Medium (glucose- and 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.
Glucose Uptake Assay Kit-Blue is not applicable for microplate assay.

UP01: Glucose Uptake Assay Kit-Blue
UP02: Glucose Uptake Assay Kit-Green
UP03: Glucose Uptake Assay Kit-Red
Revised on March 15, 2023

Preparation of Solutions

1. Preparation of Probe stock solution

Add 40 µl of DMSO to the Glucose Uptake Probe tube and dissolve by pipetting and vortex mixing.
Note: Store the Probe stock solution at -20°C, protect from light. The Probe stock solution is stable for 1 month.

2. Preparation of Probe solution

Dilute Probe stock solution 500-fold* with glucose- and serum-free medium.
*The optimum concentration of Probe solution varies depending on cell types.
Please refer to the list of cell types on the product HP to determine the optimal concentration (dilution range: x250 - x1000).

	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	2000 µl
WI Solution (50x)	120 µl	24 µl	12 µl	120 µl	40 µl

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

Preliminary experiments

Optimization of measurement conditions

Optimization of the measurement conditions is strongly recommended since glucose uptake capacity varies with cell type. When you use this product for the first time, if you are measuring different cell types, please refer to the following general experiments shown below.

- Competitive inhibition of the probe uptake with glucose (see Experimental Example 1)*1
- Inhibition of the probe uptake with glucose transporter inhibitors (see Experimental Example 2)*2

*1 In some cell types, the cellular probe uptake may not be competitively inhibited with glucose due to differences in expression levels or types of glucose transporters. Please refer to the Q&A on the product web page for technical tips.
Q, What should I do if competitive inhibition of cellular probe uptake with glucose is not possible?
*2 Please refer to the Q&A on the product web page for glucose transporter inhibitor results.

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, and incubate at 37°C for 15 minutes*2 in a 5% CO_2 incubator.
5. Remove the supernatant and wash the cells with ice-cold WI Solution (1x) twice.
6. Remove the supernatant, add ice-cold WI Solution (1x), and incubate at room temperature for 5 minutes.*3
7. Remove the supernatant and add ice-cold WI Solution (1x).
8. Observe the cells under a fluorescence microscope.*4 *5

*1 Pre-warm the culture medium and Probe solution in an incubator (37°C). Glucose uptake into the cells may be affected by the temperature of the culture medium and Probe solution.
*2 The optimum staining time varies depending on cell types. Please refer to the list of cell types on the product HP to determine the optimal time (time range: 5 – 60 minutes).
*3 This step is necessary to avoid a high background.
*4 When using adherent cells for flow cytometry, prepare a cell suspension using trypsin or a cell scraper.
*5 When the background fluorescence is high, repeat the washing steps 6 - 7.

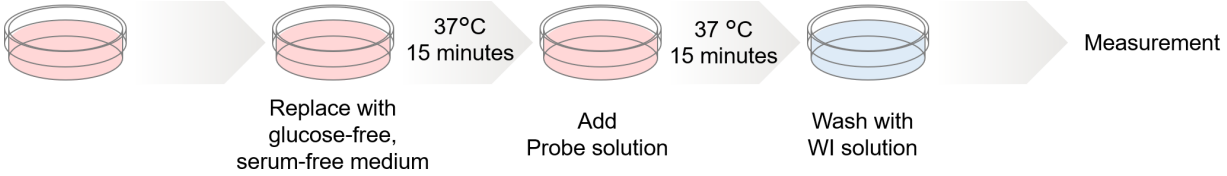


Figure 3. Protocol of Glucose Uptake Assay Kit

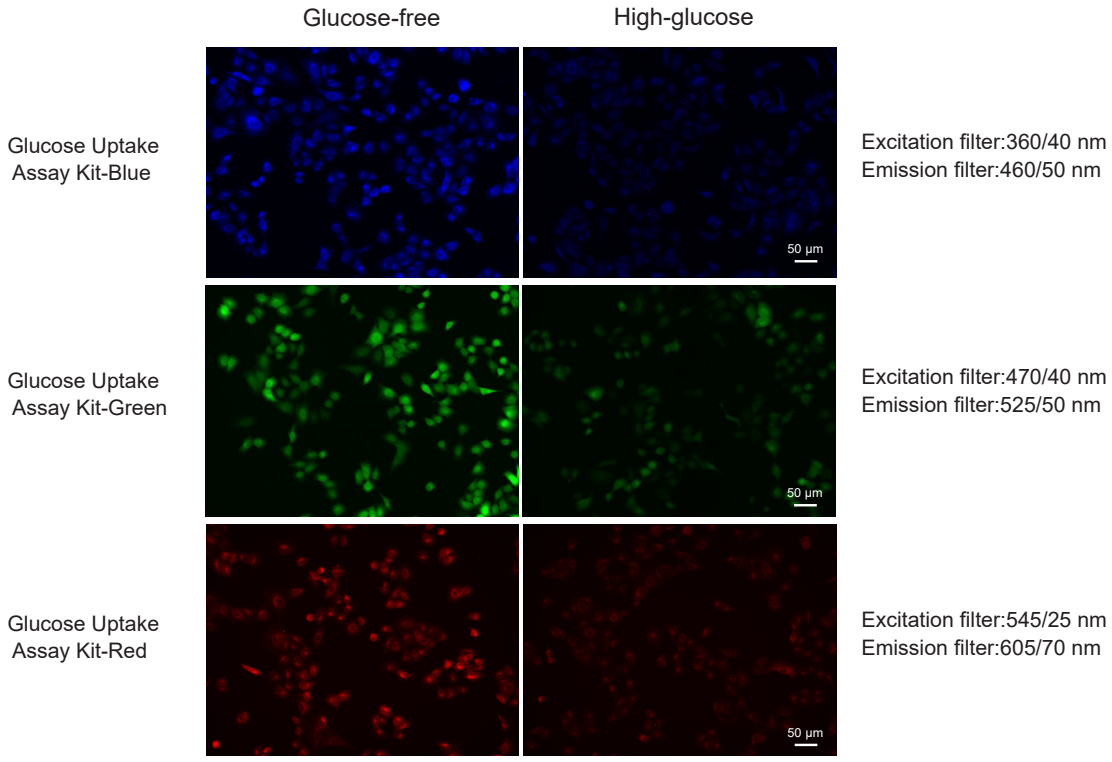
Experimental Example 1

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

1-1. Fluorescence microscopy and microplate reading

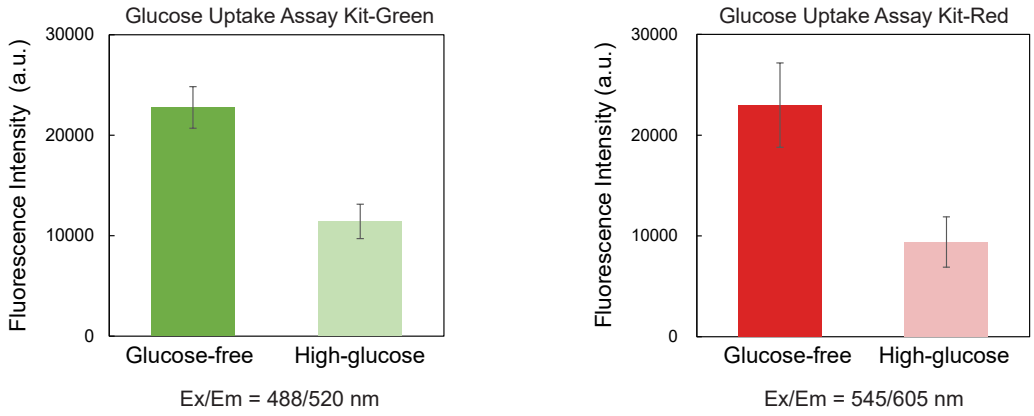
1. A549 cells (1.0×10^5 cells/ml, 150 µl) in MEM (10% FBS) were seeded in a 96-well microplate (ib89626 : ibidi GmbH) and cultured at 37°C overnight in a 5% CO_2 incubator.
2. After removing the supernatant, the cells were washed twice with 150 µl of DMEM (glucose- and serum-free, 37°C).
3. DMEM (150 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 (BZ-X700, Keyence Corporation) and the fluorescence intensity was measured with a microplate reader (Infinite m200 PRO, Tecan Trading AG, bottom reading).

(a)



Glucose-free: DMEM (glucose- and serum-free)
High-glucose: DMEM (high-glucose, serum-free)

(b)

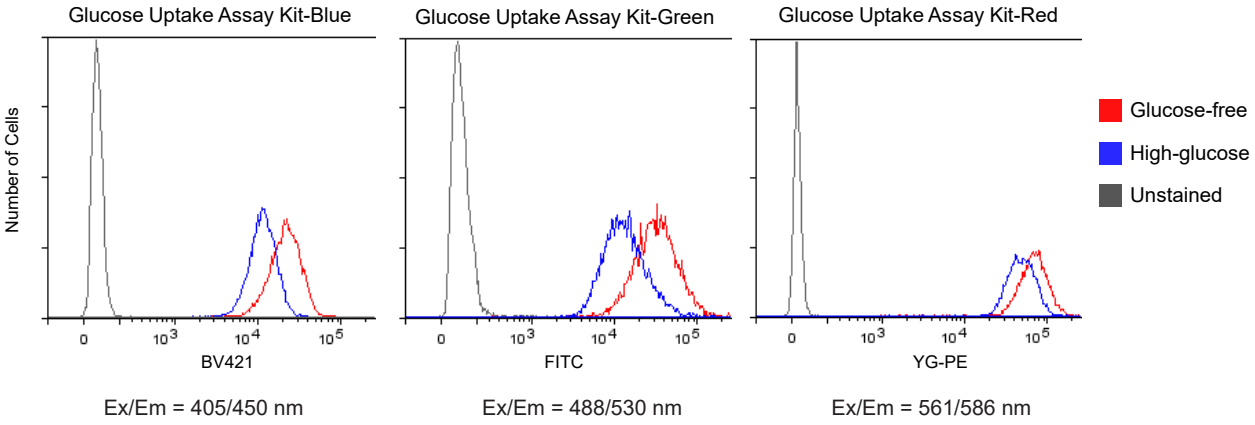


Glucose-free: DMEM (glucose- and serum-free)
High-glucose: DMEM (high-glucose, serum-free)

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

1-2. Flow cytometry

1. A549 cells (1.33×10^5 cells/ml, 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 (LSR-Fortessa X-20, Becton, Dickinson and Company).



Ex/Em = 405/450 nm　　Ex/Em = 488/530 nm　　Ex/Em = 561/586 nm

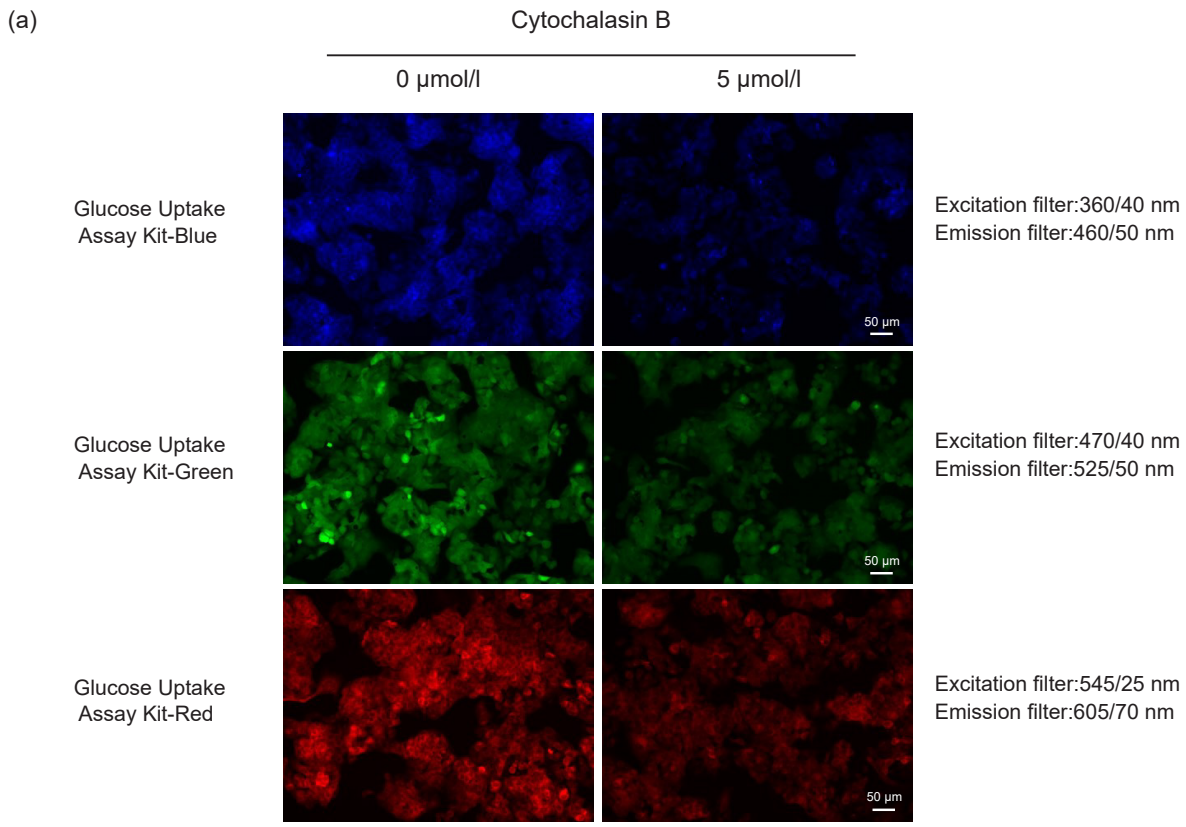
Glucose-free: DMEM (glucose- and serum-free)
High-glucose: DMEM (high-glucose, serum-free)

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

Experimental Example 2

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

1. HepG2 cells (3.0 × 10^5 cells/ml, 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, 37°C) 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- and 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 (BZ-X700, Keyence Corporation) and the fluorescence intensity was measured with a microplate reader (Infinite m200 PRO, Tecan Trading AG, top reading).



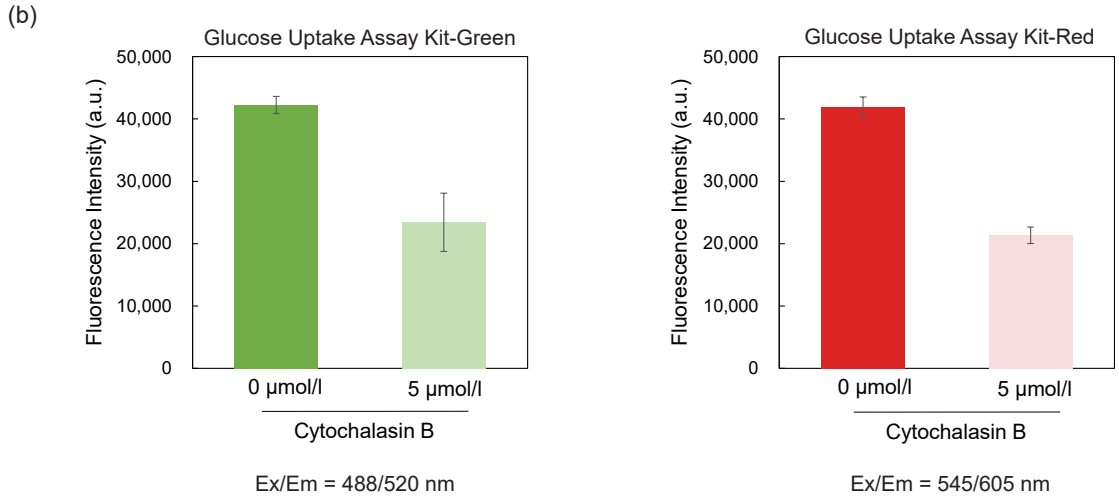


Figure 6. Inhibition of the probe uptake by Cytochalasin B (HepG2 cells)
(a) Fluorescence imaging, (b) plate reading.

Experimental Example 3

Measurement of increased uptake glucose level followed by adipocyte differentiation

-Adipocyte differentiation-

1. 3T3-L1 cells (1.0×10^5 cells/ml, 150 µl) in DMEM (10% FBS) were seeded in a 96-well microplate (ib89626: ibidi GmbH) and cultured at 37°C 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, 150 µl) 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 (BZ-X700, Keyence Corporation).

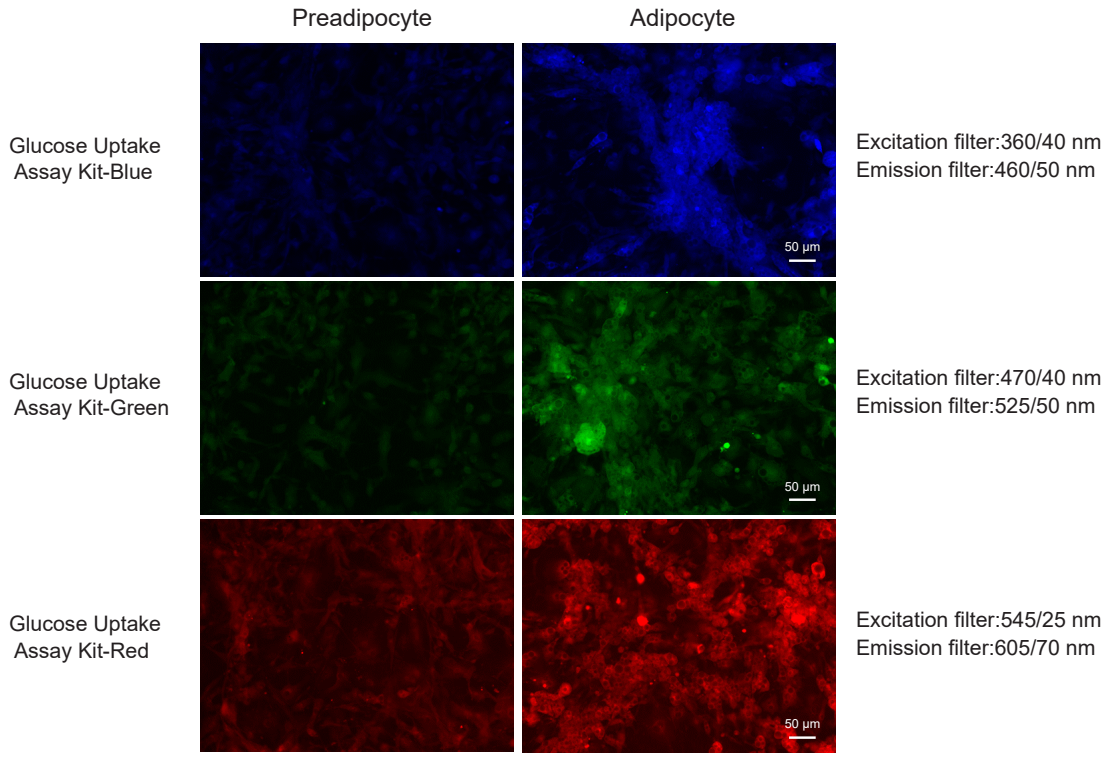


Figure 7. Enhancement of glucose uptake by differentiation

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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