Fatty Acid Uptake Assay Kit

Technical Manual

General Information

Fatty acids are important biological energy sources. Meanwhile, fatty acid uptake is related to diseases such as diabetes and obesity and is a metabolic marker in cancer cells. Because cancer cells, which actively proliferate require a large quantity of lipids, fatty acid synthesis and uptake are actively carried out. Therefore, many drugs have been developed that target the fatty acid metabolism pathways of cancer cells[1)].

The fatty acid analog included in this kit is taken up into cells via fatty acid transporters. The kit enables analysis of the fatty acid uptake ability of cells using fluorescence imaging, microplate measurement, and flow cytometry. In addition, a reagent that eliminates the fluorescence of any extracellular fatty acid analog is included, so it is possible to easily measure the actual fatty acid uptake capability even if washing the cells is difficult.

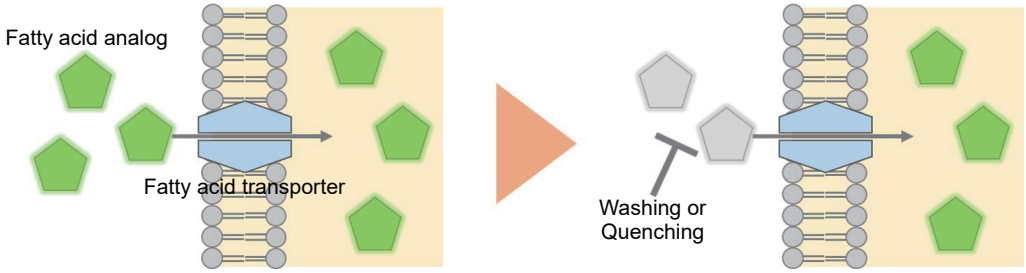


Figure 1. Principle of Fatty Acid Uptake Assay Kit

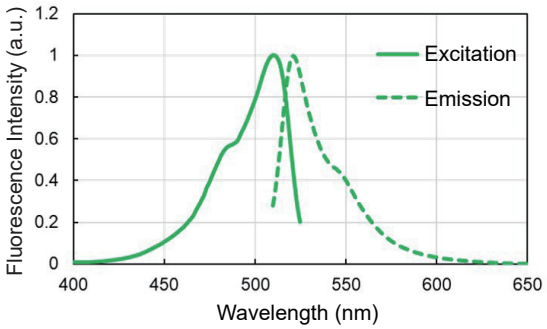


Figure 2. Excitation and emission spectra of Fatty Acid Uptake Probe

Kit Contents

Fatty Acid Uptake Probe	×1
Quenching Buffer (Red cap)	11 ml×1
Washing Buffer (10×)	11 ml×1

Storage Conditions

Store at 0−5 °C

Required Equipment and Materials

- Medium (serum-free)
- Fluorescence microscope, fluorescence microplate reader, or flow cytometer
- 20−200 µl multichannel pipette
- 100−1000 µl, 20−200 µl, 2−20 µl micropipettes
- Dimethyl sulfoxide (DMSO)
- Conical tube
- 1.5-ml microtube

Precautions

- Equilibrate reagents to room temperature prior to use.
- Briefly centrifuge the tube before opening to drop the content to the bottom of the cap.
- Please refer to Table 1 for suitable fluorescence wavelengths for each application.

Table 1. Recommended filter settings

Fluorescence microplate reader	Fluorescence microscope	Flow cytometer
Excitation 480−520 nm Emission 515−560 nm	Confocal microscope: Excitation 488 nm Emission 500−550 nm Fluorescence microscope: GFP or FITC filter	Excitation 488 nm FITC filter

Measurement Method Selection Guide

Refer to Table 2. Choose either Washing Buffer (10×) or Quenching Buffer depending on the cell type and experimental conditions.

Table 2. Selection guide for Washing Buffer (10×) or Quenching Buffer

○ : measurable, × : unmeasurable, △ : refer to notes (※)

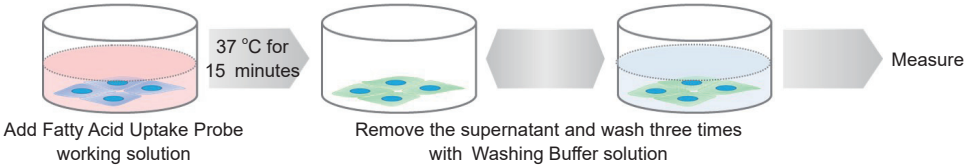
		Washing Buffer (10×)		Quenching Buffer	
		Adherent cells	Suspension cells	Adherent cells	Suspension cells
Necessity of washing		necessary		unnecessary	
Microplate reader	Bottom reading (clear bottom)	○		○	△※1
	Top reading	○		×	
Fluorescence microscope		○		○	
Confocal microscope		○		△※2	
Flow cytometer		○		×	

※ 1 It is possible to measure the fatty acid uptake ability by seeding the cells (approximately 3×10^5 cells/well). Allow them to settle to the bottom of the microplate for a while.

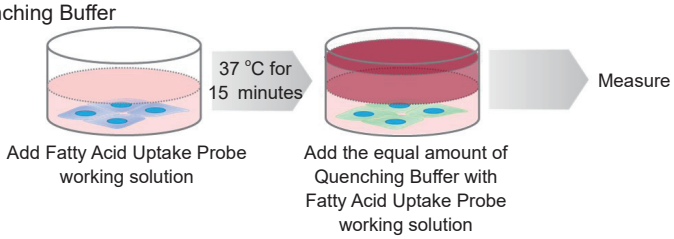
※ 2 When performing transmitted light observation under a confocal microscope, a 488-nm laser cannot be used. For transmitted light observation, please dilute Quenching Buffer 10-fold with Washing Buffer solution or use a 640-nm laser.

※ More information is posted on the product web page.

(a) Using Washing Buffer (10×)



(b) Using Quenching Buffer



Preparation of Solutions

Preparation of Fatty Acid Uptake Probe stock solution

Add 24 µl DMSO to the Fatty Acid Uptake Probe tube and dissolve by pipetting.

※ The Fatty Acid Uptake Probe stock solution is stable for 1 month when stored -20 °C.

※ The dye might deposit in the tube due to the storage and shipping conditions, but this does not affect the performance and experimental results, so please use it as it is.

Preparation of Fatty Acid Uptake Probe working solution

Dilute Fatty Acid Uptake Probe stock solution 500-fold with a serum-free medium.

※ Refer to Table 3.

※ Please use up Fatty Acid Uptake Probe working solution within that day.

Table 3. Examples of Fatty Acid Uptake Probe working solution (per well)

	Adherent cells				Suspension cells
Culture equipment (amount)	6-well (1.5 ml/well)	24-well (0.3 ml/well)	96-well (0.1 ml/well)	35-mm dish (1.5 ml/well)	1.5-ml microtube (0.5 ml/tube)
serum-free medium	1500 µl	300 µl	100 µl	1500 µl	500 µl
Fatty Acid Uptake Probe stock solution	3 µl	0.6 µl	0.2 µl	3 µl	1 µl

Preparation Washing Buffer solution

Dilute Washing Buffer (10×) 10-fold with ddH_2O.

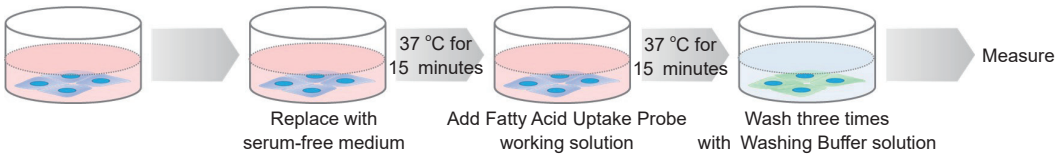
※ Refer to Table 4.

※ Please use up the Washing Buffer solution within that day.

Table 4. Required amount of Washing Buffer solution by vessel type

	Adherent cells				Suspension cells
Culture equipment	6-well	24-well	96-well	35-mm dish	1.5-ml microtube
Washing Buffer solution	1.5 ml/well	300 µl/well	200 µl/well	1.5 ml/well	500 µl/tube

Fluorescence imaging and microplate reading — Using Washing Buffer solution



〈For adherent cells〉
(1) Seed cells in a dish or microplate.
(2) Remove the culture medium and wash the cells twice with a serum-free medium.
(3) Add a serum-free medium and incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(4) Remove the supernatant and add Fatty Acid Uptake Probe working solution.
(5) Incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(6) Remove the supernatant and wash the cells three times with the Washing Buffer solution.※
(7) Add Washing Buffer solution and observe the cells under a fluorescence microscope or measure the fluorescence intensity with a fluorescence microplate reader.
※ If the background fluorescence is high, repeat the washing.

〈For suspension cells〉
(1) Prepare a cell suspension in a 1.5-ml microtube.
(2) Centrifuge at 300×*g* for 5 minutes and remove the supernatant.
(3) Add a serum-free medium and resuspend the pellet by pipetting; centrifuge at 300×*g* for 5 minutes, and remove the supernatant. Repeat this step twice.
(4) Add serum-free medium and resuspend the pellet by pipetting; incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(5) Centrifuge at 300×*g* for 5 minutes and remove the supernatant.
(6) Add Fatty Acid Uptake Probe working solution and resuspend the pellet by pipetting; incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(7) Centrifuge at 300×*g* for 5 minutes and remove the supernatant.
(8) Add the Washing Buffer solution and resuspend the pellet by pipetting; centrifuge at 300×*g* for 5 minutes, and remove the supernatant. Repeat this step three times.※
(9) Add the Washing Buffer solution and observe the cells under a fluorescence microscope or measure the fluorescence intensity with a fluorescence microplate reader.
※ If the background fluorescence is high, repeat the washing.

Fluorescence imaging and microplate reading — Using Quenching Buffer

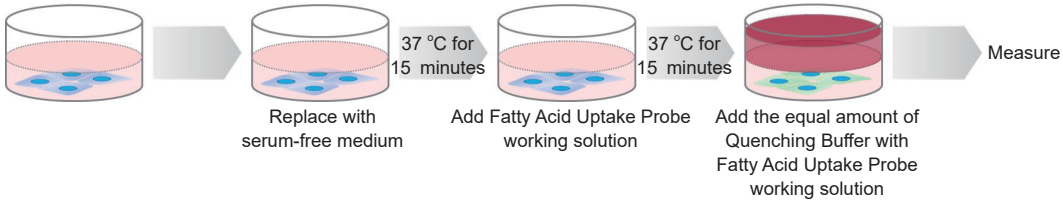


Table 5. Required amount of Quenching Buffer by vessel type

	Adherent cells				Suspension cells
Culture equipment	6-well	24-well	96-well	35-mm dish	1.5-ml microtube
Washing Buffer solution	1.5 ml/well	300 µl/well	200 µl/well	1.5 ml/well	500 µl/tube

〈For adherent cells〉
(1) Seed cells in a dish or microplate.
(2) Remove the culture medium and wash the cells twice with a serum-free medium.
(3) Add a serum-free medium and incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(4) Remove the supernatant and add Fatty Acid Uptake Probe working solution.
(5) Incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(6) Add the equal amount of Quenching Buffer with Fatty Acid Uptake Probe working solution, and observe the cells under a fluorescence microscope or measure the fluorescence intensity with a microplate reader (bottom reading).

〈For suspension cells〉
(1) Prepare a cell suspension in a 1.5-ml microtube.
(2) Centrifuge at 300×*g* for 5 minutes and remove the supernatant.
(3) Add a serum-free medium and resuspend the pellet by pipetting; centrifuge at 300×*g* for 5 minutes, and remove the supernatant. Repeat this step twice.
(4) Add a serum-free medium and resuspend the pellet by pipetting; incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(5) Centrifuge at 300×*g* for 5 minutes and remove the supernatant.
(6) Add Fatty Acid Uptake Probe working solution and resuspend the pellet by pipetting; incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(7) Add the equal amount of Quenching Buffer with Fatty Acid Uptake Probe working solution, and observe the cells under a fluorescence microscope.
※ For microplate reading using suspension cells, we recommend the method “Using Washing Buffer solution.”

Flow cytometry

〈For adherent cells〉
(1) Seed cells in a dish or microplate.
(2) Remove the culture medium and wash the cells twice with a serum-free medium.
(3) Add a serum-free medium and incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(4) Remove the supernatant and add Fatty Acid Uptake Probe working solution.
(5) Incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(6) Remove the supernatant and wash the cells three times with the Washing Buffer solution.
(7) Harvest the cells using a cell scraper or trypsinization.
(8) Analyze the cells using a flow cytometer.

〈For suspension cells〉
(1) Prepare a cell suspension in a 1.5-ml microtube.
(2) Centrifuge at 300×*g* for 5 minutes and remove the supernatant.
(3) Add a serum-free medium and resuspend the pellet by pipetting; centrifuge at 300×*g* for 5 minutes, and remove the supernatant. Repeat this step twice.
(4) Add a serum-free medium and resuspend the pellet by pipetting; incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(5) Centrifuge at 300×*g* for 5 minutes and remove the supernatant.
(6) Add Fatty Acid Uptake Probe working solution and resuspend the pellet by pipetting; incubate for 15 minutes in an incubator (37 °C, 5% CO_2).
(7) Centrifuge at 300×*g* for 5 minutes and remove the supernatant.
(8) Add the Washing Buffer solution and resuspend the pellet by pipetting; centrifuge at 300×*g* for 5 minutes, and remove the supernatant. Repeat this step three times.
(9) Analyze the cells using a flow cytometer.

Experimental Example

Inhibition of fatty acid transporter by CB-2 (HepG2 cells)

〈Fluorescence imaging and microplate reading〉
(1) HepG2 cells were seeded in a 96-well black microplate (clear bottom, 1×10^4 cells/well in DMEM containing 10% fetal bovine serum and 1% penicillin-streptomycin) and cultured overnight in an incubator (37 °C, 5% CO_2).
(2) The medium was removed, and the cells were washed twice with 200 µl of DMEM (serum-free).
(3) DMEM (100 µl, serum-free) containing 0 or 20 µmol/l CB-2 was added, and the cells were incubated for 15 minutes in an incubator (37 °C, 5% CO_2).
(4) After removing the supernatant, 100 µl of Fatty Acid Uptake Probe working solution in DMEM (serum-free) was added, and the cells were incubated for 15 minutes in an incubator (37 °C, 5% CO_2).
(5) Quenching Buffer (100 µl) was added.
(6) The cells were observed under a fluorescence microscope (BZ-X800, Keyence Corporation), and the fluorescence intensity was measured with a microplate reader (Infinite M200 PRO, Tecan Trading AG, bottom reading).

〈Flow cytometry〉
(1) HepG2 cells were seeded in a 6-well microplate (5×10^5 cells/well in DMEM containing 10% fetal bovine serum and 1% penicillin-streptomycin) and cultured overnight in an incubator (37 °C, 5% CO_2).
(2) The medium was removed, and the cells were washed twice with 1.5 ml of DMEM (serum-free).
(3) DMEM (1.5 ml, serum-free) containing 0 or 20 µmol/l CB-2 was added, and the cells were incubated for 15 minutes in an incubator (37 °C, 5% CO_2).
(4) After removing the supernatant, 1.5 ml of Fatty Acid Uptake Probe working solution in DMEM (serum-free) was added, and the cells were incubated for 15 minutes in an incubator (37 °C, 5% CO_2).
(5) After removing the supernatant, the cells were washed three times with 1.5 ml of Washing Buffer solution.
(6) The cells were harvested using a cell scraper and analyzed with a flow cytometer (FACSCanto II, Becton, Dickinson and Company).

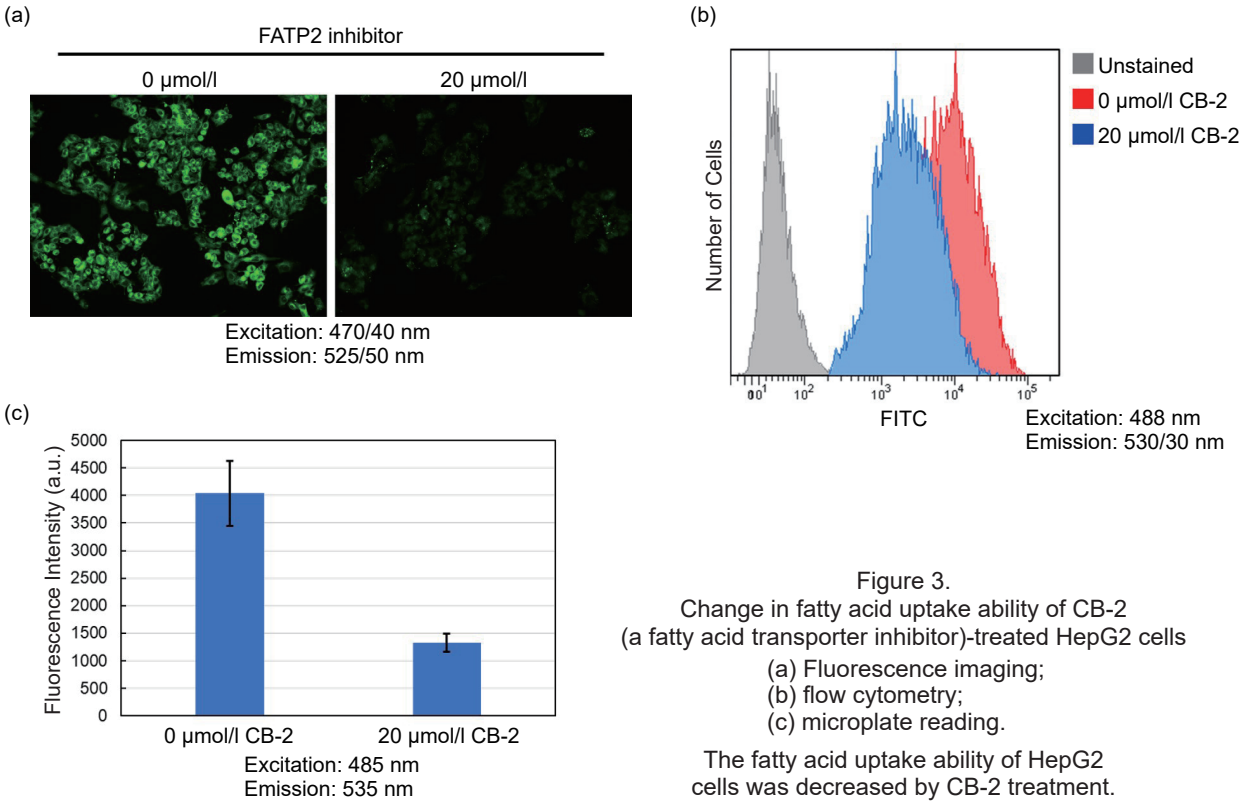


Figure 3.
Change in fatty acid uptake ability of CB-2 (a fatty acid transporter inhibitor)-treated HepG2 cells
(a) Fluorescence imaging;
(b) flow cytometry;
(c) microplate reading.

The fatty acid uptake ability of HepG2 cells was decreased by CB-2 treatment.

Reference

1) M. Zaoui, et al., *Cancers*, **2019**, *11*, 2012.

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