

General Information

Lipid droplets (LDs) are composed of neutral lipids such as triacylglycerol and cholesteryl esters surrounded by a phospholipid monolayer, and are found not only in adipocytes¹⁾ but also ubiquitously in eukaryotic organisms. Although LDs were originally considered to be a type of lipid storage machinery, a recent study has shown that LDs play an important role in regulating lipid metabolism, autophagy²⁾ and cellular senescence.³⁾ Lipi probes are small molecules that emit strong fluorescence in a hydrophobic environment such as LDs, which can be observed without any washing steps after staining with Lipi probes.⁴⁾

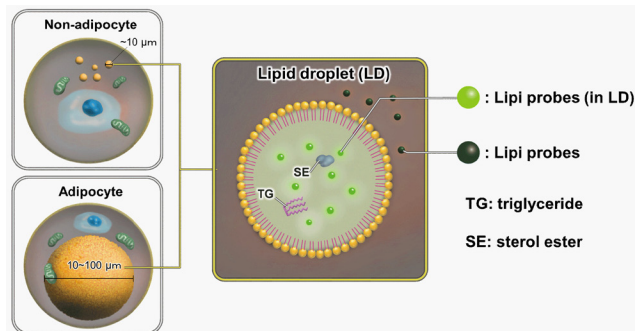


Figure 1. Staining mechanism of Lipi probes

Contents

LD01	Lipi-Blue	10 nmol x 1
LD02	Lipi-Green	10 nmol x 1
LD03	Lipi-Red	100 nmol x 1
LD04	Lipi-Deep Red	10 nmol x 1

Note: The material supplied for each dye is sufficient for 50 tests when a 35 mm dish is used. (final concentration of Lipi-Blue Lipi-Green, and Lipi-Deep Red: 0.1 μ mol/l, Lipi-Red: 1 μ mol/l)

Storage Condition

- LD01 Store in a cool and dark place.
- LD02 Store in a cool and dark place.
- LD03 Store in a cool and dark place.
- LD04 Store in a cool and dark place.

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- PBS
- Micropipettes

Fluorescent Properties

Fluorescent properties of Lipi probes

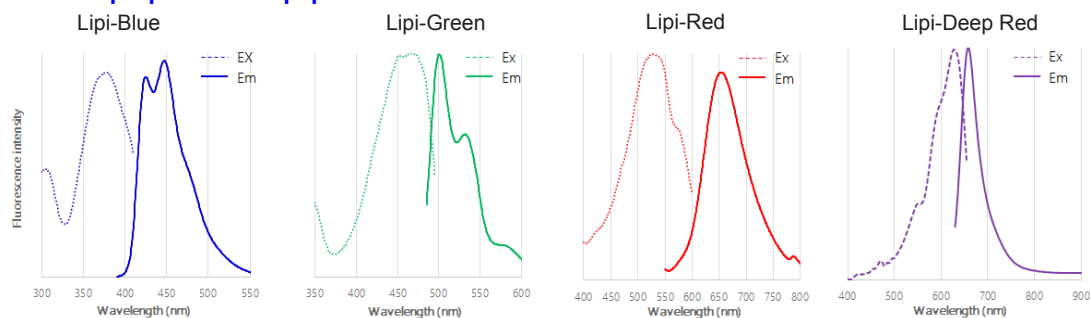


Figure 2. Excitation and emission spectra of Lipi-Blue, Lipi-Green, Lipi-Red, and Lipi-Deep Red

Preparation of Solutions

Preparation of Lipi probe DMSO stock solution

- Lipi-Blue 0.1 mmol/l DMSO stock solution: Add 100 μ l of DMSO to a tube of Lipi-Blue and dissolve by vortex mixer.
- Lipi-Green 0.1 mmol/l DMSO stock solution: Add 100 μ l of DMSO to a tube of Lipi-Green and dissolve by vortex mixer.
- Lipi-Red 1 mmol/l DMSO stock solution: Add 100 μ l of DMSO to a tube of Lipi-Red and dissolve by vortex mixer.
- Lipi-Deep Red 0.1 mmol/l DMSO stock solution: Add 100 μ l of DMSO to a tube of Lipi-Deep Red and dissolve by vortexing.

Note: Store the DMSO stock solution at -20 $^{\circ}$ C. The DMSO stock solution is stable at -20 $^{\circ}$ C for 1 month.

Note: Lipi-Blue is difficult to see because it is present in a small amount and is a colorless foam. Please prepare the Lipi-Blue DMSO stock solution carefully by vortexing with DMSO as described in the protocol.

Preparation of Lipi probe working solution

- Lipi-Blue working solution: Dilute the DMSO stock solution with PBS or serum-free medium to prepare 0.1–0.5 μ mol/l working solution.
- Lipi-Green working solution: Dilute the DMSO stock solution with PBS or serum-free medium to prepare 0.1–0.5 μ mol/l working solution.
- Lipi-Red working solution: Dilute the DMSO stock solution with PBS or serum-free medium to prepare 1–5 μ mol/l working solution.
- Lipi-Deep Red working solution: Dilute the DMSO stock solution with PBS or serum-free medium to prepare 0.1–0.5 μ mol/l working solution.

Note: Use the working solution within the same day of preparation.

Note: Serum-containing medium can also be used instead of serum-free medium.

- Seed cells on a dish for assay. Culture the cells at 37 °C overnight in a 5% CO₂ incubator.
- Remove the culture medium and wash the cells with PBS twice.
- Add the Lipi series working solution and incubate at 37 °C for 30 minutes in the 5% CO₂ incubator.
Note: When using epifluorescence microscope, replace the working solution with a culture medium or a buffer to reduce the fluorescence background.
- Observe the sample under a fluorescence microscope.

Note: Following filter sets are recommended.

Lipi-Blue: Excitation 405 nm, Emission 450–500 nm

Lipi-Green: Excitation 488 nm, Emission 500–550 nm

Lipi-Red: Excitation 561 nm, Emission 565–650 nm

Lipi-Deep Red: Excitation 640 nm, Emission 650–700 nm

Note: If no fluorescent signal was observed, please try followings.

- Increase the magnification of the fluorescence microscope in case the lipid droplets are small
- Increase the incubation time by 1–2 h.
- Increase the reagent concentration (increase Lipi-Blue, Lipi-Green, and Lipi-Deep Red to 1 μmol/l, increase Lipi-Red to 10 μmol/l)
- Prepare lipid droplet-containing cells as a positive control for comparison with the samples. The positive control can be prepared by incubating cells with a 200 μmol/l oleic acid-containing culture medium overnight.

Usage Examples

Induction of LDs formation using oleic acid (HeLa cells)

- HeLa cells were seeded on a μ-slide 8-well plate and cultured at 37 °C overnight in a 5% CO₂ incubator.
- The supernatant was removed and the cells were washed twice with serum-free medium.
- Oleic acid (200 μmol/l)-containing medium (DMEM/10% FBS/1% PBS) was added to the each well, and the cells were cultured at 37 °C overnight in a 5% CO₂ incubator.
- The supernatant was removed and the cells were washed twice with serum-free medium.
- The lipi working solution was added and the cells were incubated at 37 °C for 30 min in a 5% CO₂ incubator.
- The cells were observed using a fluorescence microscope.

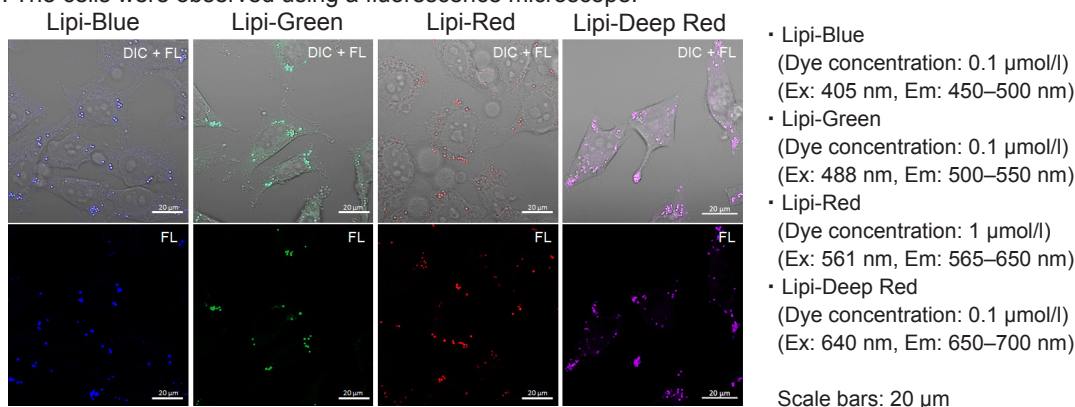
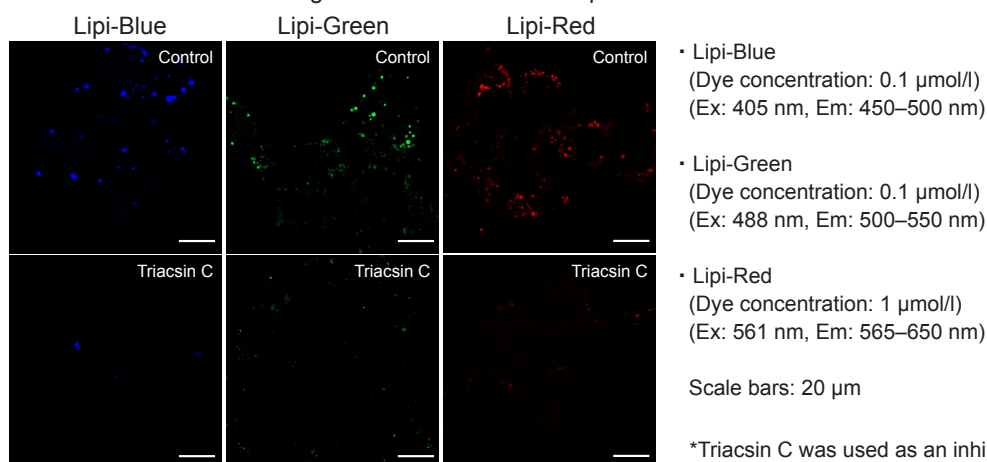


Figure 3. Fluorescent images of oleic acid treated HeLa cells

Inhibition of LDs formation using Triacsin C (HepG2 cells)

- HepG2 cells were seeded on a μ-slide 8-well plate and cultured at 37 °C overnight in a 5% CO₂ incubator.
- The supernatant was removed and the cells were washed twice with serum-free medium.
- Triacsin C prepared with serum-containing medium (5 μmol/l) was added to the each well, and the cells were cultured at 37 °C overnight in a 5% CO₂ incubator.
- The supernatant was removed and the cells were washed twice with serum-free medium.
- Lipi working solution was added and the cells were incubated at 37 °C for 30 min in a 5% CO₂ incubator.
- The cells were observed using a fluorescence microscope.



*Triacsin C was used as an inhibitor for LD formation

Figure 4. Fluorescent images of Triacsin C treated HepG2 cells

References

- Fujimoto, T. et al., *Histochem Cell Biol.*, **2008**, 130(2), 263–279.
- Singh, R. et al., *Nature*, **2009**, 458(7242), 1131–1135.
- Yokoyama, M. et al., *Cell Reports*, **2014**, 7(5), 1691–1703.
- Tatenaka, Y. et al., *Biochemistry.*, **2019**, 58(6), 499–503.

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LD01: Lipi-Blue
LD02: Lipi-Green
LD03: Lipi-Red
LD04: Lipi-Deep Red