Lipi-Blue
Lipi-Green
Lipi-Red

Technical Manual

General Information

Lipid droplets (LDs) are composed of neutral lipids such as triacylglycerol and cholesteryl ester that are surrounded by phospholipid monolayers, and are to be seen ubiquitously not only in adipocytes[1]. Although LDs were simply considered as a lipid storage machinery, a recent study has stated that LDs play an important role in regulating lipid metabolism, autophagy[2] and cellular senescence[3]. Lipi probes are small molecule which emit strong fluorescence in hydrophobic environment such as in LDs. LDs can be observed without any washing steps after staining with Lipi probes.

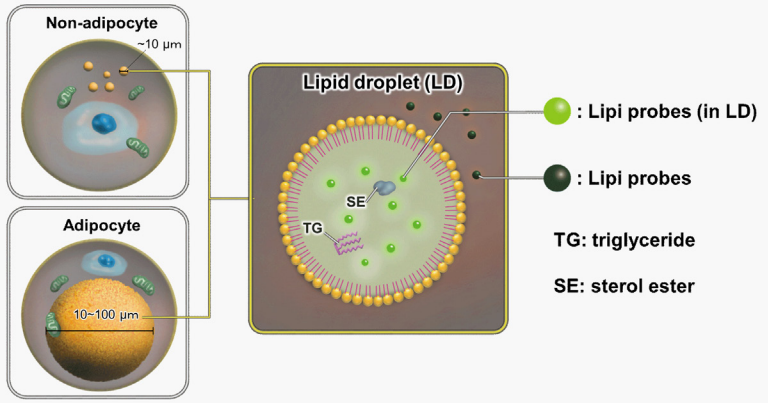


Figure 1. Staining mechanism of Lipi probes

Contents

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

*Equivalent to 50 tests when 35 mm dish is used. (final concentration of Lipi-Blue and Lipi-Green: 0.1 μmol/l, Lipi-Red: 1 μmol/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.

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- PBS
- Micropipettes

Fluorescent properties

Fluorescent properties of Lipi probes

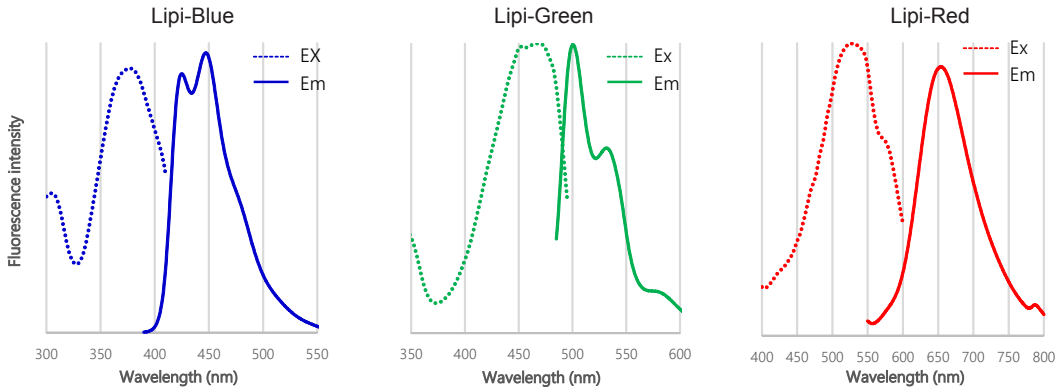


Figure 2. Excitation and emission spectra of Lipi-Blue, Lipi-Green, and Lipi-Red

Preparation of Solutions

Preparation of Lipi probe DMSO stock solution

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

Note: Store the DMSO stock solution at -20 °C. The DMSO stock solution is stable at -20 °C for 1 month.
Note: Lipi-Blue is difficult to see due to its small amount and colorless foam.
Please prepare a Lipi-Blue DMSO stock solution carefully by vortex mixer 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 μmol/l working solution.
- Lipi-Green working solution:
  Dilute the DMSO stock solution with PBS or serum-free medium to prepare 0.1–0.5 μmol/l working solution.
- Lipi-Red working solution:
  Dilute the DMSO stock solution with PBS or serum-free medium to prepare 1–5 μmol/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.

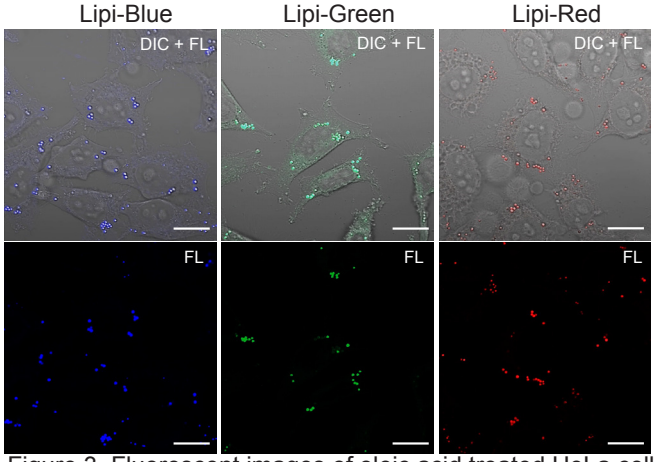
General protocol

1. Seed cells on a dish for assay. Culture the cells at 37 °C overnight in a 5% CO_2 incubator.
2. Remove the culture medium and wash the cells with PBS twice.
3. Add Lipi series working solution and incubate at 37 °C for 30 minutes in the 5% CO_2 incubator.
   Note: When using epifluorescence microscope, replace the working solution with a culture medium or a buffer to reduce the fluorescence background.
4. 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
   Note: If no fluorescent signal was observed, please try followings.
   1. Lipid droplets are too small, increase the magnification of the fluorescence microscope.
   2. Increase the incubation time 1-2 hours.
   3. Increase the reagent concentration. (Lipi-Blue and Lipi-Green: 1 µmol/L, Lipi-Red: 10 µmol/L)
   4. Prepare lipid droplet-containing cells as a positive control to compare with samples. The positive control can be prepared by the incubation with a 100 µmol/L oleic acid-containing culture medium overnight.

Usage examples

Induction of LDs formation using oleic acid (HeLa cells)

1. HeLa cells were seeded on a µ-slide 8 well plate and cultured at 37 °C overnight in a 5% CO_2 incubator.
2. The supernatant was removed and the cells were washed with serum-free medium twice.
3. Oleic acid (200 µmol/l) contained medium (DMEM/ 10% FBS/ 1% PS) was added to the each well, and the cells were cultured at 37 °C overnight in the 5% CO_2 incubator.
4. The supernatant was removed and the cells were washed with serum-free medium twice.
5. Lipi working solution was added and the cells were incubated at 37 °C for 30 minutes in the 5% CO_2 incubator.
6. The cells were observed under a fluorescence microscope.



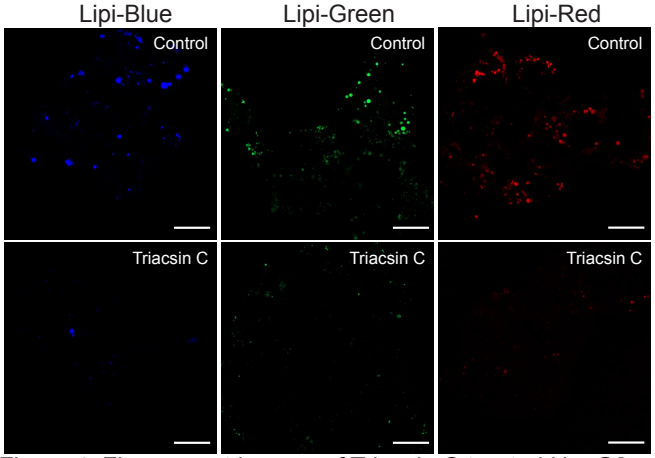
- Lipi-Blue
  (Dye concentration: 0.1 µmol/L)
  (Ex: 405 nm, Em: 450–500 nm)
- Lipi-Green
  (Dye concentration: 0.1 µmol/L)
  (Ex: 488 nm, Em: 500–550 nm)
- Lipi-Red
  (Dye concentration: 1 µmol/L)
  (Ex: 561 nm, Em: 565–650 nm)

Scale bars: 20 µm

Figure 3. Fluorescent images of oleic acid treated HeLa cells

Inhibition of LDs formation using Triacsin C (HepG2 cells)

1. HepG2 cells were seeded on a µ-slide 8 well plate and cultured at 37 °C overnight in a 5% CO_2 incubator.
2. The supernatant was removed and the cells were washed with serum-free medium twice.
3. 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 the 5% CO_2 incubator.
4. The supernatant was removed and the cells were washed with serum-free medium twice.
5. Lipi working solution was added and the cells was incubated at 37 °C for 30 minutes in the 5% CO_2 incubator.
6. The cells were observed under a fluorescence microscope.



- Lipi-Blue
  (Dye concentration: 0.1 µmol/L)
  (Ex: 405 nm, Em: 450–500 nm)
- Lipi-Green
  (Dye concentration: 0.1 µmol/L)
  (Ex: 488 nm, Em: 500–550 nm)
- Lipi-Red
  (Dye concentration: 1 µmol/L)
  (Ex: 561 nm, Em: 565–650 nm)

Scale bars: 20 µm

*Triacsin C was used as an inhibitor for LD formation

Figure 4. Fluorescent images of Triacsin C treated HepG2 cells

References

1) Fujimoto, T. et al., *Histochem Cell Biol.*, **2008**, *130*(2), 263–279.
2) Singh, R. et al., *Nature*, **2009**, *458*(7242), 1131–1135.
3) Yokoyama, M. et al., *Cell Reports*, **2014**, *7*(5), 1691–1703.

If you need more information, please contact Dojindo technical service.

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