Lipid Droplet Assay Kit - Blue
Lipid Droplet Assay Kit - Deep Red

Technical Manual

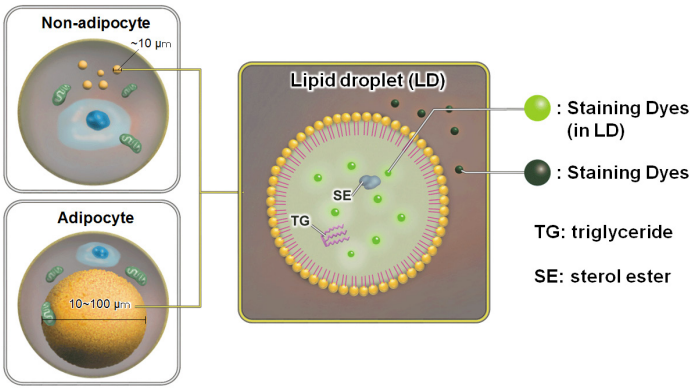
Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/LD05.pdf

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 adipocytes but also ubiquitously in eukaryotic organisms.[1] 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, autophagy[2], cellular senescence[3], and differentiation.[4]

Oil Red O and Nile Red staining are widely used to detect LDs. The method by using Oil Red O has the following disadvantages: 1) time-consuming and complicated procedure, 2) Oil Red O tends to precipicate in solution, 3) precipitated Oil Red O may cause errors of measurement, and 4) only applicable for fixed cells. On the other hand, the method by using Nile Red partelly overcome the disadvantages of Oil Red O. However, Nile Red is not well-suite method for lipid droplet assay due to its high background.

Unlike Oil Red O and Nile Red, the Lipid Droplet Assay Kit - Blue and Deep Red enable selective and easy measuring of LDs as per the enclosed protocol. The Staining Dye in this kit minimizes background due to more selective staining ability to LDs than Nile Red. In addition, the Loading Buffer solution contained in this kit maintains the health of the cells during assays. This kit can be used for live and fixed cells in flowcytometry analysis and plate reader assay.



Kit Contents

LD05 Lipid Droplet Assay Kit - Blue		LD06 Lipid Droplet Assay Kit - Deep Red	
Staining Dye - Blue	x 1	Staining Dye - Deep Red	x 1
Loading Buffer (10x)	6 ml x 1	Loading Buffer (10x)	6 ml x 1

Storage Condition

Store at 0–5 °C.

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- Medium or HBSS
- Micropipettes
- Microtube

Preparation of Solutions

Preparation of the DMSO stock solution

Add 100 µl of DMSO to a tube containing Staining Dye and dissolve it by pipetting.
Store the DMSO stock solution at -20 °C.

*Protect the DMSO stock solution from light.
*The DMSO stock soltuion is stable at -20 °C for up to a month.

Preparation of the Loading Buffer solution

Dilute the Loading Buffer (10×) 10 times using double-deionized water.

*Please use the diluted solution on the same day of preparation.

Preparation of the working solution

Dilute the DMSO stock solution 200-fold with the Loading Buffer solution to prepare a working solution.

*Please use the diluted solution on the same day of preparation.

General Protocol

The dye staining

1. Prepare cells for the assay.
2. Discard the supernatant and wash the cells twice with HBSS.
3. Add an appropriate volume of the working solution to the wells.
4. Incubate the cells at 37 °C for 1–2 h.
5. Discard the supernatant and wash the cells twice with HBSS.
6. Add HBSS and detect the cells using flow cytometer or plate reader.

*Please refer recomended filter settings bellow.
Use of other filters may cause fluorescence signal decreasing.

Table 1 Suitable filters for microplate assay

Lipid Droplet Assay Kit - Blue		
No.	Excitation filter	Emission filter
1	360 nm (20)	430 nm (35)
2	360 nm (20)	460 nm (20)
3	380 nm (20)	430 nm (35)
4	380 nm (20)	460 nm (20)

Lipid Droplet Assay Kit - Deep Red		
No.	Excitation filter	Emission filter
1	590 nm (20)	635 nm (35)
2	590 nm (20)	670 nm (25)
3	620 nm (10)	670 nm (25)

Table 2 Suitable filters for flow cytometry

Lipid Droplet Assay Kit - Blue	
Excitation laser	Emission filter
405 nm	425-475 nm

Lipid Droplet Assay Kit - Deep Red	
Excitation laser	Emission filter
640 nm	650-670 nm

Usage Examples

Lipid droplet detection of triacsin C- or oleic acid-treated HepG2 cells.

1. HepG2 cells (5×10^3 cells/well) in DMEM (10% fetal bovine serum, 1% penicillin-streptomycin) were seed on a 98-well black plate and were cultured at 37 °C in a 5% CO_2 incubator overnight.
2. The cells were washed twice with HBSS.
3. DMEM (10% fetal bovine serum, 1% penicillin-streptomycin) containing 200 µmol/l oleic acid or 5 µmol/l triacsin C was added, and cultured at 37 °C in a 5% CO_2 incubator overnight.
4. The cells were washed twice with HBSS.
5. The working solution was added to each well, and then the cells were cultured at 37 °C for 2 h in a 5% CO_2 incubator.
6. The cells were washed twice with HBSS.
7. HBSS was added, and the cells were measured using a plate reader.

*Oleic acid was used as an inducer of LDs.

*Triacsin C was used as an inhibitor for LD formation.

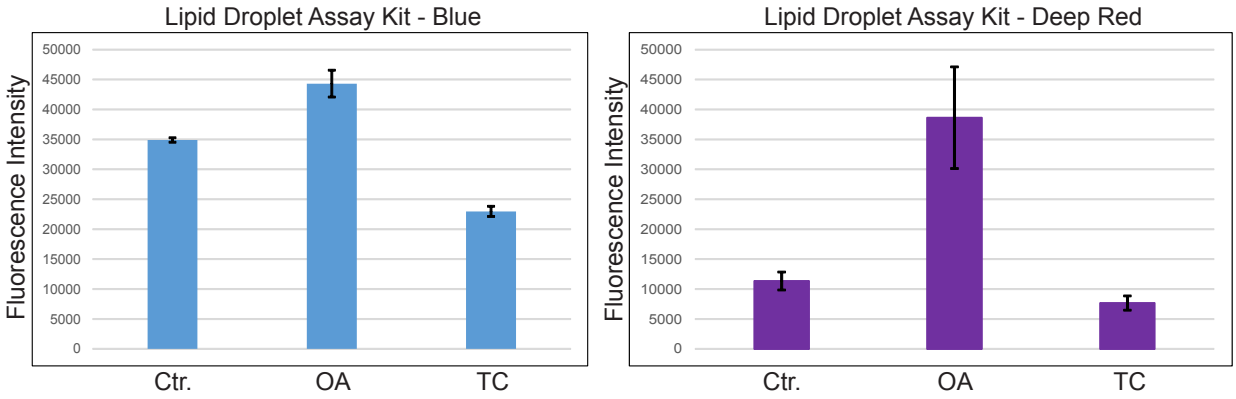


Fig. 1 Microplate assay of lipid droplets in HepG2 cells

Lipid droplet detection of triacsin C- or oleic acid-treated HeLa cells.

1. HeLa cells (2.5×10^5 cells/ml) in MEM (10% fetal bovine serum, 1% penicillin-streptomycin) containing 200 µmol/l oleic acid or 5 µmol/l triacsin C were seeded on a 6-well plate and were cultured at 37 °C in a 5% CO_2 incubator overnight.
2. The cells were washed twice with HBSS.
3. The working solution was added to each well, and then the cells were cultured at 37 °C for 2 h in a 5% CO_2 incubator.
4. The cells were washed twice with PBS.
5. The cells were tripsinized.
6. The supernatant was discarded, and HBSS was added.
7. The cells were analyzed using a flow cytometer.

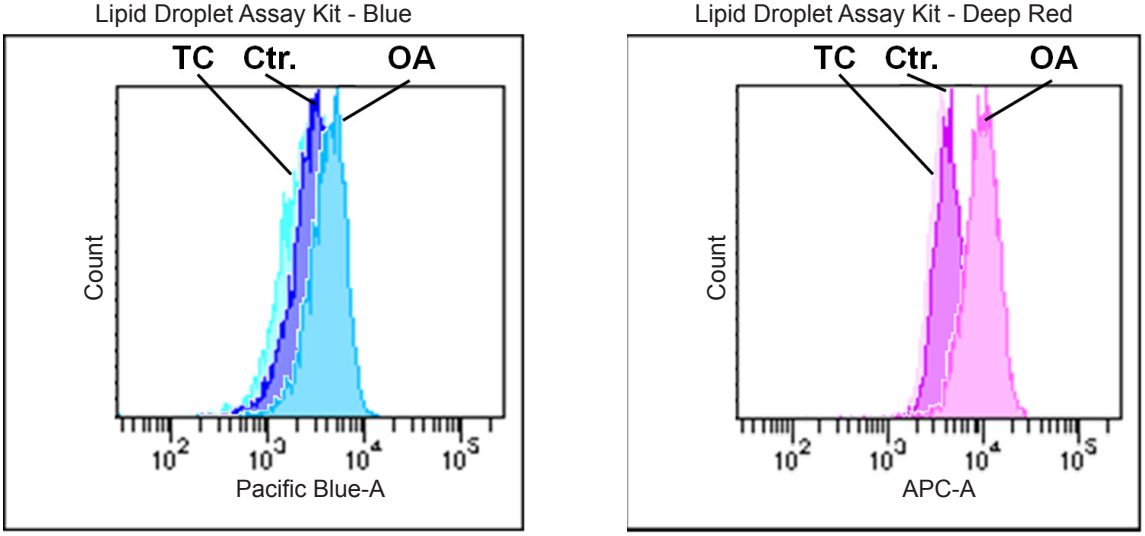


Fig. 2 Flow cytometric analysis of lipid droplets in HeLa cells

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.
4) Oka, M. et al., *Cells*, **2019**, *8*(4), 373

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

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