

Lipid Droplets Fluorescence Assay Kit

Item No. 500001



Customer Service 800.364.9897 * **Technical Support** 888.526.5351

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/ Size	Storage
600056	Lipid Droplets Assay Oleic Acid	1 vial/100 µl	-20°C
10008981	Lipid Droplets Assay Fixative (10X)	1 vial/10 ml	Room Temperature
600055	Cell-Based Assay Nile Red Solution	1 vial/50 µl	-20°C
10009322	Cell-Based Assay Buffer Tablet	3 tablets	Room Temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Adjustable pipettes and a repeating pipettor
2. A 6-, 12-, 24-, or 96-well plate for culturing cells
3. A flow cytometer, fluorescence microscope, or plate reader with the capacity to measure fluorescence using an excitation wavelength of 488 nm and an emission wavelength of 535 nm

INTRODUCTION

Background

Lipid droplets are lipid storage organelles found in the cytoplasm of most eukaryotic cells. The storage of lipids in mammalian cells was long considered to occur mainly in adipocytes and steroidogenic cells. Lipid droplets were treated as inert organelles in which excess fatty acids were converted to neutral lipids to be used for metabolism, membrane synthesis (phospholipids and cholesterol), and steroid synthesis. Further evidence demonstrates that lipid droplets are complexes composed of lipids together with PAT proteins, including Perilipin (P), adipophilin (A), and tail-interacting protein of 47 kDa (TIP 47, T). Lipid droplets are a fundamental component of intracellular lipid homeostasis in all cell types, and they can provide a rapidly mobilized lipid source for many important biological processes such as membrane trafficking and cell signaling.^{1,2} In fact, lipid droplet formation is cell and stimulus specific and is highly regulated. New lipid droplets are synthesized in the course of a variety of immunopathological conditions, especially during inflammation. The emerging role of lipid droplets as inflammatory mediators raises lipid droplets to key markers of leukocyte activation and attractive targets for novel anti-inflammatory therapies.¹

About This Assay

Cayman's Lipid Droplets Fluorescence Assay Kit can be used to study regulators of lipid droplet biogenesis in a variety of conditions, including inflammation. The main advantage of this assay is that the green fluorescence of Nile Red is both very sensitive and specific for lipid droplets.³ Furthermore, changes in lipid droplet biogenesis in various cell types in response to different manipulations can be both qualified and quantified by fluorescence microscopy, flow cytometry, or fluorescence plate readers. Simultaneous visualization of lipid droplets and associated proteins is also possible when used with an antibody conjugated to a blue fluorophore. Oleic acid, which is commonly used to induce lipid droplet formation in cultured cells, is included as a positive control. This kit provides sufficient reagents to effectively treat/stain 480 individual wells of cells when utilized in a 96-well plate format. Lower density plates will still require approximately the same amount of reagent on a per plate basis. Therefore, in effect five plates worth of cells can be examined irrespective of the number of wells/plate. Exceptions include protocols in which non-adherent cells are utilized.

Reagent Preparation

1. Assay Buffer Preparation

Dissolve each Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of distilled water. The buffer should be stable for approximately one year at room temperature.

2. Fixative Solution Preparation

Prepare a working solution by diluting the Lipid Droplets Assay Fixative (10X) (Item No. 10008981) 1:10 in the diluted Assay Buffer.

3. Nile Red Staining Solution Preparation

Thaw the Cell-Based Assay Nile Red Solution (Item No. 600055) at room temperature. Mix well. To avoid repeated freezing/thawing of this solution, we recommend that you make small aliquots and store them at -20°C.

Prepare a working solution by diluting the Nile Red Stock Solution 1:1,000 in the diluted Assay Buffer. Mix well to make sure no particles or flakes remain in the solution.

Performing the Assay

Pipetting Hints

- Nile Red is light sensitive. All staining procedures must be performed without direct exposure to intense light. Therefore, incubations need to be done in the dark.
- For all assay protocols described below, it is imperative that samples be analyzed immediately following completion of the staining.

Flow Cytometry

1. Seed cells in a 6-, 12-, or 24-well plate at a density of 10^5 - 10^6 cells/well in 2, 1, or 0.5 ml of culture medium. Culture the cells in a CO₂ incubator at 37°C for at least 24 hours before treatment.
2. The following day, treat cells with experimental compounds or vehicle for 24 hours, or for the period of time used in your typical experimental protocol. To use the included oleic acid as a positive control, dilute the Lipid Droplets Assay Oleic Acid (Item No. 600056) 1:5,000-1:100,000 into your culture medium.
3. At the end of the treatment, trypsinize (adherent cells) or collect cells (suspension cells). Centrifuge at 800 x g for five minutes to pellet the cells and wash once with 1 ml of Assay Buffer.
4. Aspirate supernatant.
5. Resuspend cell pellet at a concentration of 10^6 cells/ml in diluted Fixative Solution. Incubate cells at room temperature for ten minutes.
6. Centrifuge at 800 x g for five minutes to pellet the cells.
7. Aspirate supernatant.
8. Add 1 ml of Assay Buffer to each sample. Mix well.
9. Centrifuge at 800 x g for five minutes to pellet the cells.
10. Aspirate supernatant.

11. Resuspend cell pellet at a concentration of 10^6 cells/ml in the Nile Red Staining Solution. It is important to achieve a monodisperse cell suspension at this step by pipetting up and down repeatedly.
12. Incubate cells at room temperature for 10-15 minutes. Protect samples from the light.
13. Centrifuge at 800 x g for five minutes to pellet the cells.
14. Aspirate the supernatant.
15. Add 1 ml of Assay Buffer to each sample. Mix well.
16. Centrifuge at 800 x g for five minutes to pellet the cells.
17. Aspirate the supernatant.
18. Resuspend cell pellet in 0.5-1 ml of Assay Buffer depending on cell number. Pipette up and down repeatedly to achieve a monodisperse cell suspension. Cells can now be analyzed in the FL1 channel of a flow cytometer with a 488 nm excitation laser.

Fluorescence Microscopy

A 6-, 12-, 24-, or 96-well culture plate can be used for this method. We recommend that the cell density be $\leq 1 \times 10^6$ cells/ml. Optimal conditions will be dependent on the cell type.

1. Seed cells in a 6-, 12-, 24-, or 96-well plate at a density of 10^4 - 10^6 cells/well in 2, 1, 0.5, or 0.1 ml of culture medium. Culture the cells in a CO₂ incubator at 37°C for at least 24 hours before treatment.
2. The following day, treat cells with experimental compounds or vehicle for 24 hours, or for the period of time used in your typical experimental protocol. To use the included oleic acid as a positive control, dilute the Oleic Acid stock solution (Item No. 600056) 1:5,000-1:100,000 into your culture medium.
3. Centrifuge the plate at 800 x g for five minutes.
4. Aspirate the supernatant.
5. Add 2, 1, 0.5, or 0.1 ml of the Assay Buffer to each well of the 6-, 12-, 24-, or 96-well plate.
6. Centrifuge the plate at 800 x g for five minutes.
7. Aspirate the supernatant.
8. Add 1, 0.5, 0.25, or 0.05 ml of diluted Fixative Solution to each well of the 6-, 12-, 24-, or 96-well plate. Incubate the cells at room temperature for 10 minutes.

9. Centrifuge the plate at 800 x g for five minutes.
10. Aspirate the supernatant.
11. Add 2, 1, 0.5, or 0.1 ml of Assay Buffer to each well of the 6-, 12-, 24-, or 96-well plate.
12. Centrifuge the plate at 800 x g for five minutes.
13. Aspirate the supernatant.
14. Add 1, 0.5, 0.25, or 0.05 ml of the Nile Red Staining Solution to each well of the 6-, 12-, 24-, or 96-well plate.
15. Incubate cells at room temperature for 10-15 minutes. Protect samples from the light.
16. Centrifuge the plate at 800 x g for five minutes.
17. Aspirate the supernatant. Add 2, 1, 0.5, or 0.1 ml of Assay Buffer to each well of the 6-, 12-, 24-, or 96-well plate. Examine lipid droplet staining with a fluorescence microscope equipped with a filter designed to detect FITC (excitation/emission = 485/535). The lipid droplets should appear as green round dots.

Plate Reader Fluorescence Detection

A 96-well **BLACK** culture plate should be used for this method. We recommend that cell density be $\leq 1 \times 10^5$ cells/well. Optimal conditions will be dependent on the cell type.

1. Culture cells in a black clear bottom 96-well plate at a density of 5×10^4 - 1×10^5 cells/well. Culture the cells in a CO₂ incubator at 37°C for at least 24 hours before treatment.
2. The following day, treat cells with experimental compounds or vehicle for 24 hours, or for the period of time used in your typical experimental protocol. To use the included oleic acid as a positive control, dilute the Lipid Droplets Assay Oleic Acid (Item No. 600056) 1:5,000-1:100,000 into your culture medium. Include wells for a background control (see step 14, on page 10).
3. Centrifuge the plate at 800 x g for five minutes.
4. Aspirate the supernatant.
5. Add 0.1 ml of Assay Buffer to each well.
6. Centrifuge the plate at 800 x g for five minutes.
7. Aspirate the supernatant.

8. Add 0.05 ml of Fixative Solution to each well. Incubate the cells at room temperature for 10 minutes.
9. Centrifuge the plate at 800 x g for five minutes.
10. Aspirate the supernatant.
11. Add 0.1 ml of Assay Buffer to each well.
12. Centrifuge the plate at 800 x g for five minutes.
13. Aspirate the supernatant.
14. Add 0.05 ml of the Nile Red Staining Solution to each well except background control wells. Incubate cells at room temperature for 10-15 minutes. Protect samples from the light.
15. Centrifuge the plate at 800 x g for five minutes.
16. Aspirate the supernatant.
17. Add 0.2 ml of Assay Buffer to each well.
18. Centrifuge the plate at 800 x g for five minutes.
19. Aspirate the supernatant.
20. Add 0.1 ml of Assay Buffer to each well. Read the fluorescence intensity of lipid droplets with a fluorescence plate reader using an excitation at 485 nm and an emission at 535 nm.

PERFORMANCE CHARACTERISTICS

Cell Staining

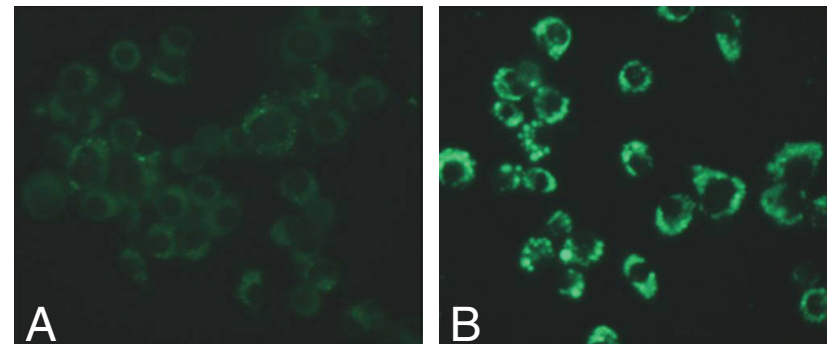


Figure 1. Oleic acid dramatically induces lipid droplet accumulation in neuro-2a cells. Neuro-2a cells were plated at a density of 2×10^4 cells/well in a black clear-bottom 96-well plate and grown overnight. The next day, cells were treated with vehicle (control, *Panel A*) or 400 μ M oleic acid (treated, *Panel B*). On the third day, cells were processed for lipid droplet staining according to the protocol described in this booklet. In cells cultured with MEM only, there were a few cells with tiny lipid droplets (*Panel A*). When cells were fed with 400 μ M oleic acid overnight, most of cells accumulated numerous large lipid droplets.

Plate Reader Fluorescence Detection

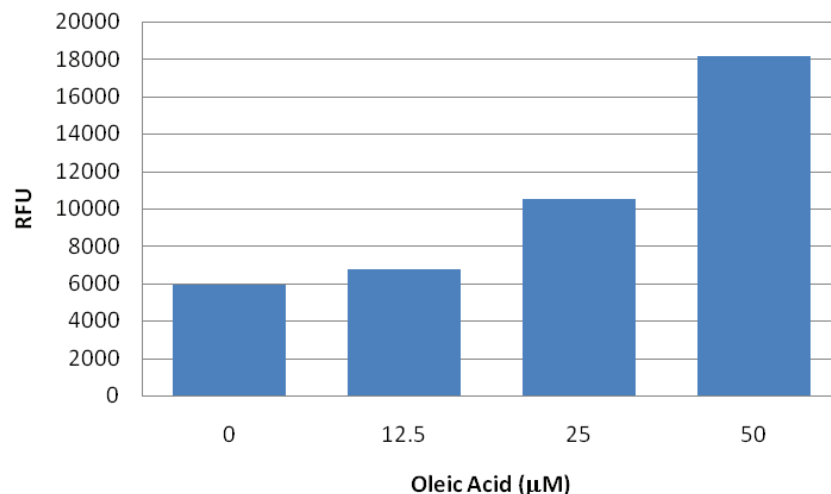


Figure 2. Oleic acid increases lipid droplet fluorescence staining intensity in Raw 264.7 cells. Raw 264.7 cells were plated at a density of 10^4 cells/well in a black clear-bottom 96-well plate and grown overnight. The next day, cells were treated with vehicle or different concentration of oleic acid for six hours and then processed for lipid droplet staining according to the protocol described in this booklet. Fluorescence intensity of lipid droplets was measured with a plate reader using a filter designed for fluorescein measurement (excitation/emission = 485 nm/535 nm).

Flow Cytometry

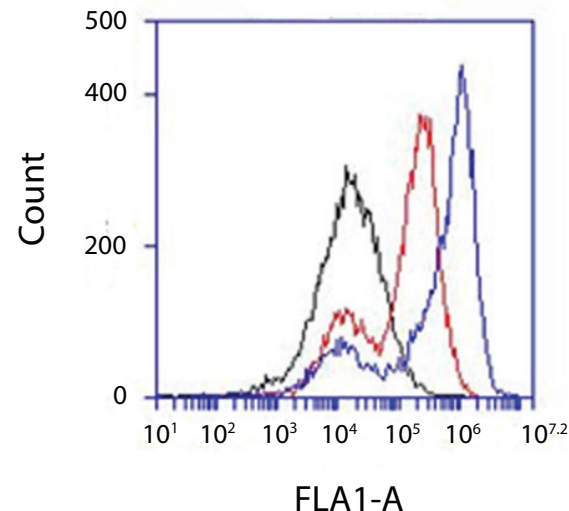


Figure 3. Oleic acid dramatically increases lipid droplet accumulation in Jurkat cells. Jurkat cells were plated at a density of 1×10^6 cells/well in a 6-well plate and grown overnight. The next day, cells were treated with vehicle (black line), 100 μM oleic acid (red line), or 400 μM oleic acid (blue line). On the third day, cells were processed for lipid droplet staining, according to the protocol described in this booklet. Cells were analyzed with an Accuri™ C6 Flow Cytometer. Oleic acid treatment at 100 μM caused a significant increase in lipid droplet accumulation, and 400 μM oleic acid treatment caused even more lipid droplet accumulation compared to that of control, as evidenced by a significant shift of the cell population to higher fluorescence.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No staining or poor staining	Cells are not healthy or dead	Use only healthy cells
No difference among treatments, including positive control	A. Cells detach from the plate, thus losing cells during process B. Lipid droplets burst during process	A. Use lower concentration of compound, or shorten treatment time B. Use cell types that have low lipid droplet content under normal conditions
High or low fluorescence intensity in all treatments, including control	Used wrong filter, such as a filter for red fluorescence	Use filter for green fluorescence

References

1. D'Avila, H., Maya-Monteiro, C.M., and Bozza, P.T. Lipid bodies in innate immune response to bacterial and parasite infections. *International Immunopharmacology* **8**, 1308-1315 (2008).
2. Martin, S. and Parton, R.G. Lipid droplets: A unified view of a dynamic organelle. *Molecular Cell Biology* **7**, 373-378 (2006).
3. Listenberger, L.L. and Brown, D.G. Fluorescent detection of lipid droplets and associated proteins. *Current Protocols in Cell Biology Supplement* **35**, 24.2.1-24.2.11 (2007).

Related Products

Adipogenesis Assay Kit - Item No. 10006908
 Adipolysis Assay Kit - Cat No. 10009381
 p-Aminohippuric Acid (PAH) Assay Kit - Item No. 700880
 Cholesterol Uptake Cell-Based Assay Kit - Item No. 600440
 Creatine Kinase Fluorometric Assay Kit - Item No. 700630
 Glycerol Cell-Based Assay Kit - Cat No. 10011725
 Glycogen Assay Kit - Item No. 700480
 Inulin Fluorometric Assay Kit - Item No. 700770
 Leptin (human) Standard Set - Item No. 400604
 Lipase Activity Assay Kit - Item No. 700640
 Malate Fluorometric Assay Kit - Item No. 700790
 Steatosis Colorimetric Assay Kit - Item No. 10012643

Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

NOTES

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