Lipid Droplets Fluorescence Assay Kit

Item No. 500001

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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	RT
600055	Cell-Based Assay Nile Red Solution	1 vial/50 µl	-20°C
10009322	Cell-Based Assay Buffer Tablet	3 tablets	RT

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 RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling.Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

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 2, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A 96-well plate for culturing/staining cells (black, clear bottom for plate reader or polypropylene v-bottom for flow cytometric staining)
2. 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

About This Assay

Cayman's Lipid Droplets Fluorescence Assay Kit can be used to study regulators of lipid droplet biogenesis. The main advantage of this assay is that the green fluorescence of Nile Red is both very sensitive and specific for lipid droplets.[1] 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 fluorescent plate readers. Simultaneous visualization of lipid droplets and associated proteins is also possible when used with antibodies conjugated to compatible fluorophores. 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.

PRE-ASSAY PREPARATION

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.

ASSAY PROTOCOL

Performing the Assay

NOTES

- 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.
- The following protocol can be used for staining cells to be assayed by flow cytometry (best for suspension cells), microscopy, or plate reader detection. Volumes are optimized for 96-well plate staining, though other plate sizes can be used by scaling as necessary.

1. Culture cells as your experiment requires: for plate reader detection, a black clear-bottom 96-well plate is recommended; for flow cytometry, any size wells can be used for culture. For a positive control, the included Lipid Droplets Assay Oleic Acid (Item No. 600056) can be added at a final dilution of 1:2,000 to 1:10,000 (depending on application) into the culture medium for 24 hours. While optimal cell numbers must be determined for each application, cells should not be more than 80% confluent by the end of the assay.
2. For flow cytometric applications, at the end of the treatment, collect cells into wells of a polypropylene 96-well staining plate and continue with the staining protocol. For microscopy and plate readers, stain the cells in the plate in which they are cultured.

3. Centrifuge at 400 x g for five minutes.
4. Aspirate supernatant.
5. Add 0.1 ml of Assay Buffer to each well.
6. Centrifuge the plate at 400 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 400 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 400 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 400 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 400 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 filter sets designed to detect FITC (ex/em 485/535 nm).

PERFORMANCE CHARACTERISTICS

Performance Characteristics

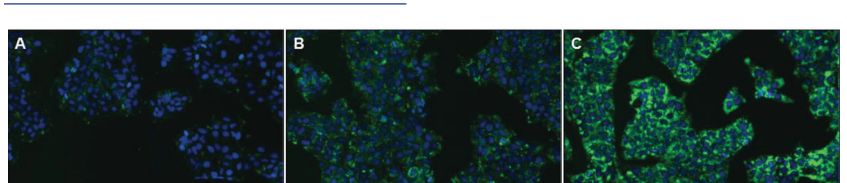

Figure 1. Oleic Acid increases lipid droplets in Huh7 cells. Huh7 cells were plated at a density of 1 x 10^4 cells/well in a 96-well plate and cultured overnight. The next day, cells were treated overnight with Lipid Droplets Assay Oleic Acid at either 1:2000 (*Panel C*) or 1:8000 (*Panel B*) or left untreated (*Panel A*) before following the staining protocol on page 10 of the kit booklet. DAPI (Item No. 601361) was included at a dilution of 1:600 in the Nile Red Staining Solution to stain the nuclei, aiding in visualization. Cells were imaged using BioTek's Cytation™ 5 Cell Imaging Multi-Mode Reader. The 10X objective and two LED filter cubes were used: Violet (blue nuclear stain) and FITC (lipid droplets).

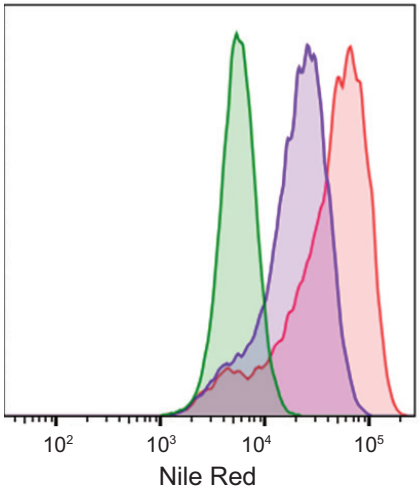

Figure 2. Oleic acid induces lipid droplet accumulation in Jurkat cells as measured by flow cytometry. Jurkat cells were plated at $1x10^5$ cells/well and treated overnight with Lipid Droplets Assay Oleic Acid at 1:4,000 (red) or 1:8,000 (purple), or with no treatment (green).

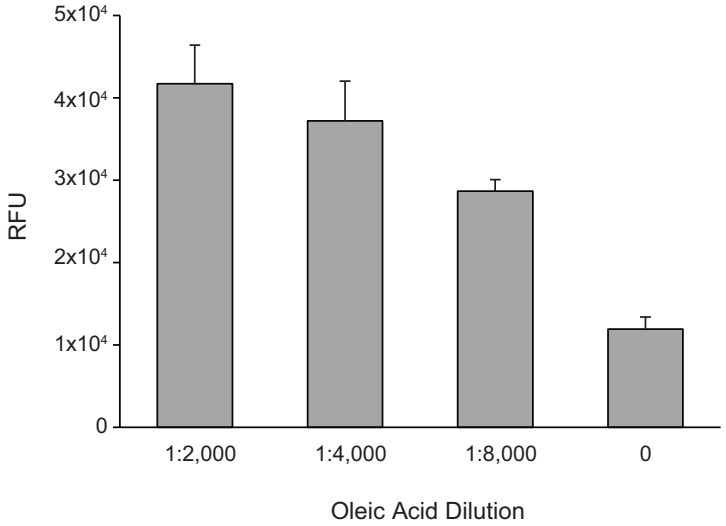

Figure 3. Oleic acid increases lipid droplet accumulation in RAW264.7 cells RAW264.7 cells were plated at $8x10^4$ cells/well and allowed to adhere. Lipid Droplets Assay Oleic Acid was added overnight to the indicated concentrations.

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

Reference

1. 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).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.