Cell Navigator™ Fluorimetric Lipid Droplet Assay Kit *Red Fluorescence*

Catalog number: 22735
Unit size: 200 Tests

Component	Storage	Amount
Component A: Droplite™ Red	Freeze (< -15 °C), Minimize light exposure	1 vial (40 µL, 500 X in DMSO)
Component B: Staining Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (20 mL)

OVERVIEW

Lipid droplets, also referred to as lipid bodies, oil bodies or adiposomes, are lipid-rich cellular organelles that regulate the storage and hydrolysis of neutral lipids. They also serve as a reservoir of lipid source for many important biological processes such as fatty acid and cellular cholesterol for energy and membrane formation and maintenance. Abnormal accumulation of the cytoplasmic lipid droplets occurs in a variety of pathological conditions and can be an indicator of metabolic deficiency or pathogenesis. AAT Bioquest's Cell Navigator™ Fluorimetric Lipid Droplet Assay Kit is a robust tool that could quantitatively measure lipid droplet accumulation. Droplite™ Red is used in the kit for lipophilic stain. Droplite™ Red is intensely fluorescent in a lipid-rich environment while it has minimal fluorescence in aqueous media. It is an excellent vital stain for the detection of intracellular lipid droplets with fluorescence microscopy, flow cytometry or fluorescence microplate reader. The red fluorescence signal could be read observed using the filter set of TRITC.

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds
2. Add 100 µL Droplite™ Red staining solution
3. Incubate at room temperature or 37°C for 10 to 30 min
4. Read fluorescence intensity at Ex/Em = 550/640 nm (Cutoff = 610 nm), image cells using fluorescence microscope with TRITC filter or flow cytometer with FL1 channel

Important Following is our recommended protocol for live cells. This protocol only provides a guideline, and should be modified according to your specific needs. Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microscope

Excitation	TRITC filter set
Emission	TRITC filter set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	550 nm
Emission	640 nm
Cutoff	610 nm
Recommended plate	Solid black

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Droplite™ Red staining solution
Dilute 2 µL of Droplite™ Red (Component A) to 1 mL of Staining Buffer (Component B). Protect from light. *Note: 20 µL of Droplite™ Red (Component A) is enough for one 96-well plate. The optimal concentration of the Droplite™ Red varies depending on specific applications. The staining conditions may be modified according to a particular cell type and the permeability of the cells or tissues to the probe.*

PREPARATION OF WORKING SOLUTION

Add 2 µL of Droplite™ Red (Component A) to 1 mL of Staining Buffer (Component B) to make Droplite™ Red staining soluton. Protect from light.

Note 20 µL of Droplite™ Red (Component A) is enough for one 96-well plate. The optimal concentration of the Droplite™ Red varies depending on specific applications. The staining conditions may be modified according to a particular cell type and the permeability of the cells or tissues to the probe.

SAMPLE EXPERIMENTAL PROTOCOL

For adherent cells:

1. Grow cells either in a 96-well black wall/clear bottom plate (100 µL/well/96-well) or on cover-slips inside a petri dish filled with the appropriate culture medium.
2. Gently aspirate the culture medium, and add equal volume (such as 100 µL/well/96-well plate) of the Droplite™ Red staining solution.
3. Incubate the cells in a 37 °C, 5% CO_2 incubator for 10 - 30 minutes.
4. Remove Droplite™ Red staining solution (Optional).
5. Read the fluorescence intensity with a microplate reader at Ex/Em =550/640 nm (Cutoff = 610 nm) or observe the cells using a fluorescence microscope with a TRITC filter set.

For suspension cells:

1. Centrifuge the cells at 1000 rpm for 5 minutes to get 1 - 5 × 10^5 cells per tube.
2. Resuspend cells in 500 µL of Droplite™ Red staining solution.
3. Incubate at room temperature or 37 °C for 10 to 30 min, protected from light.
4. Centrifuge to remove the Droplite™ Red staining solution, and resuspend cells in 500 µL of pre-warmed medium or buffer of your choice to get 1 - 5 × 10^5 cells per tube (Optional).
5. Monitor the fluorescence increase using fluorescence microscope with a TRITC filter set or flow cytometer with FL1 channel.

Note Since Droplite™ Red has minimal fluorescence in aqueous media, aspiration of the growth medium and removal of Droplite™ Red staining solution after staining is optional. Stained cells can be fixed with 3 - 4% formaldehyde. In addition, prefixed cells (3 - 4%

formaldehyde fixation) can be stained with Droplite™ Red staining solution.

EXAMPLE DATA ANALYSIS AND FIGURES

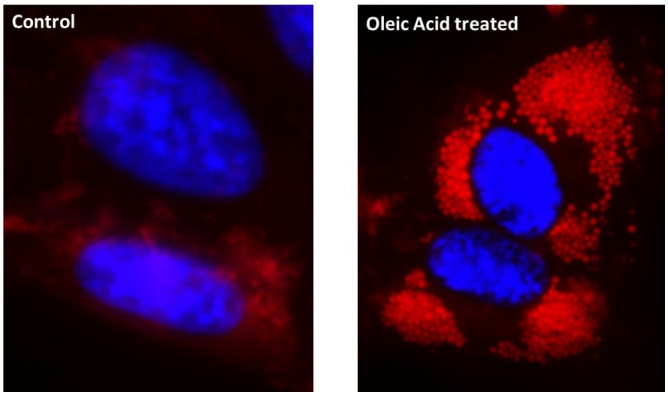

Figure 1.
Fluorescence images of intracellular lipid droplets in control (Left) and Oleic Acid treated HeLa cells (Right) using Cell Navigator™ Lipid Droplets Fluorescence Assay Kit. HeLa cells were incubated with 300 uM of Oleic Acid for 24 hours to induce intracellular lipid droplets formation. After washing with PBS, the cells were labeled with 1X Droplite™ Red and Hoechst 33342 (Cat#17533).

DISCLAIMER

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