Cell Navigator™ Fluorimetric Lipid Droplet Assay Kit
Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22735 (200 assays)	Keep in freezer and protect from light	Fluorescence microscope

Introduction

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 at Ex/Em=550/640 nm or observed using the filter set of TRITC.

Kit Components

Components	**Amount**
Component A: Droplite™ Red	40 µL (500 X in DMSO)
Component B: Staining Buffer	20 mL

Assay Protocol

Brief Summary

Prepare cells with test compounds → Add 100 µL Droplite™ Red staining solution → Incubate at room temperature or 37 °C for 10 to 30 min → Read fluorescence intensity at Ex/Em = 550/640 nm or record images using the TRITC filter set.

Note: Following is our recommended protocol for live cells. This protocol only provides a guideline, and should be modified according to your specific needs.

1. Prepare Droplite™ Red staining solution:

1.1 Warm Droplite™ Red (Component A) to room temperature.

1.2 Prepare Droplite™ Red staining solution by diluting 2 µL of Droplite™ Red (Component A) to 1 mL of Staining Buffer (Component B).

Note 1. 20 µL of Droplite™ Red (Component A) is enough for one 96-well plate. Aliquot and store unused Droplite™ Red at ≤ -20 °C. Protect it from light and avoid repeated freeze-thaw cycles.

Note 2. 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.

2. Prepare and stain cells:

2.1 For adherent cells:

a. 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.

b. Gently aspirate the culture medium, and add equal volume (such as 100 µL/well/96-well plate) of the Droplite™ Red staining solution (from Step 1.2).
c. Incubate the cells in a 37 °C, 5% CO_2 incubator for 10~30 minutes.
d. Remove Droplite™ Red staining solution (*Optional, see Note 1 below*).
e. Read Fluorescence at 550/640 nm with a microplate reader or observe the cells using a fluorescence microscope with a TRITC filter set.

2.2 For suspension cells:
a. Centrifuge the cells at 1000 rpm for 5 minutes to get 1-5 × 10^5 cells per tube.
b. Resuspend cells in 500 µL of Droplite™ Red working solution (from Step 1.2).
c. Incubate at room temperature or 37 ºC for 10 to 30 min, protected from light.
d. Centrifuge to remove the Droplite™ Red working 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, see Note 1 below*).
e. Monitor the fluorescence increase using fluorescence microscope with a TRITC filter set or flow cytometer at FL1 channel.

Note 1: Since Droplite™ Red has minimal fluorescence in aqueous media, aspiration of the growth medium (step 2.1b) and removal of Droplite™ Red staining solution (step 2.1 d) after staining is optional.
Note 2: 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.

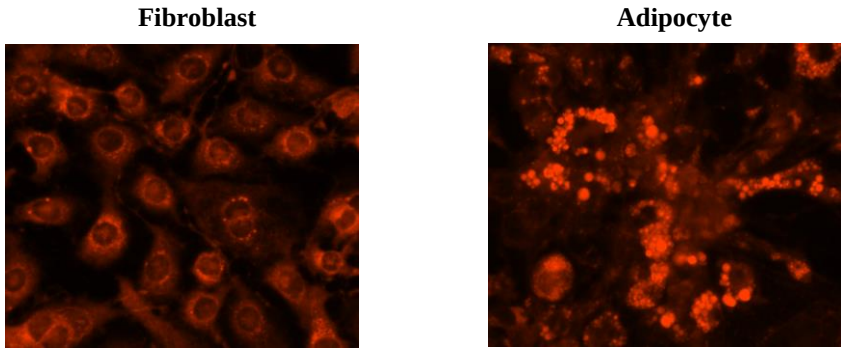

Figure 1. Fluorescence images of intracellular lipid droplets in 3T3-L1 Fibroblast (Left) and Adipocyte cells (Right) using Cell Navigator™ Lipid Droplets Fluorescence Assay Kit. The fluorescence signal was measured using fluorescence microscope with a TRITC filter.

References

1. Greenberg AS, Coleman RA, Kraemer FB, McManaman JL, Obin MS, Puri V, et al. The role of lipid droplets in metabolic disease in rodents and humans. J Clin Invest 2011;121:2102-10
2. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. J Clin Endocrinol Metab 2004;8:2595-600.