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

Catalog number: 22730
Unit size: 200 Tests

Component	Storage	Amount
Component A: Nile Green™	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL-200 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 Droplets Assay Kit is a simple assay that could quantitatively measure lipid droplet accumulation. Nile Green™ is used in the kit for lipophilic stain. Nile Green™ 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. Unlike Nile Red which has broad range of fluorescence spectrum, Nile Green™ stains intracellular lipid droplets with green fluorescence only. It can be used with other fluorescence dyes for multicolor staining. The green fluorescence signal could be observed using the filter set of FITC.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds
2. Add Nile Green™ working solution
3. Incubate at room temperature or 37°C for 10 to 30 min
4. Read fluorescence intensity with fluorescence microscope using FITC filter

Important Following is our recommended protocol for live cells. This protocol only provides a guideline, and should be modified according to your specific needs. Since Nile Green™ has minimal fluorescence in aqueous media, aspiration of the growth medium and removal of Nile Green™ 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 Nile Green™ staining solution.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	485 nm
Emission:	520 nm
Cutoff:	510 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode
Instrument:	Fluorescence microscope
Excitation:	FITC filter set
Emission:	FITC filter set
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

Prepare Nile Green™ working solution by diluting 5 µL of 200X Nile Green™ (Component A) to 1 mL of Staining Buffer (Component B).

Note 50 µL of Nile Green™ (Component A) is enough for one 96-well plate. Protect from light. The optimal concentration of the Nile Green™ 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 CELL SAMPLES

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

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 Nile Green™ staining solution.
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 10 - 30 minutes.
4. Remove Nile Green™ working solution (Optional).
5. Read Fluorescence at 485/520 nm with a microplate reader or observe the cells using a fluorescence microscope with a FITC 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 Nile Green™ working solution.
3. Incubate at room temperature or 37°C for 10 to 30 min, protected from light.
4. Centrifuge to remove the Nile Green™ 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).
5. Monitor the fluorescence increase using fluorescence microscope with a FITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

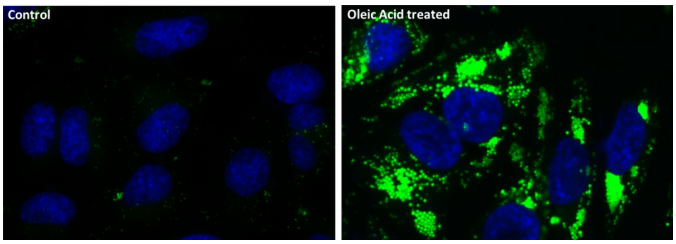

Figure 1. Fluorescence images of intracellular lipid droplets in control (Left) and

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 Nile Green™ and Hoechst 33342 (Cat#17533).

DISCLAIMER