Red Fluorometric Lipid Droplet Assay Kit

Introduction

Lipid droplets (LDs) are mainly composed of phospholipid monolayers and hydrophobic cores of neutral lipids (triglycerides and cholesterol, etc.), and are widely found in animals, plants, and bacteria. Studies have shown that lipid droplets play an important role in regulating lipid metabolism, autophagy, cell differentiation and cell senescence.

Red Fluorometric Lipid Droplet Assay Kit is a kit for the detection of intracellular lipid droplets using Nile Red. Nile Red is a fluorescent probe that can label lipid droplets. Nile Red is cell-penetrating, which can be used for staining live and fixed cells. Nile Red is virtually non-fluorescent in water or other polar solvents, and emits a strong fluorescence once combined with neutral lipids such as triglycerides. Nile Red has a wide range of emission spectrum, and it has better selectivity for staining lipid droplets when viewing with green fluorescence (450-500 nm excitation; >528 nm emission) rather than red fluorescence (515-560 nm excitation; >590 nm emission).

Oil Red O is also commonly used for lipid droplet detection. However, Oil Red O staining has problems such as complex operation, time-consuming, low accuracy, and only suitable for fixed cells. Nile Red does not have the above problems, but it is not suitable for multicolor fluorescence staining due to its wide range of emission spectrum. If multicolor fluorescence staining is required, consider using BODIPY probes.

Components and Storage

Components	K2169-100 T	K2169-500 T
Nile Red (1000x)	100 µL	500 µL
Hoechst 33342 (1000x)	100 µL	500 µL
Assay Buffer	100 mL	500 mL

Store the kit at -20°C, stable for 1 year. Nile Red (1000x) and Hoechst 33342 (1000x) should be stored away from light.

Protocol

1. **Preparation of staining solution:** For 6-well plates, refer to the following table to prepare the staining solution and mix it thoroughly. Prepare fresh staining solution every time. The concentration of Nile Red probes in the staining solution can be adjusted between 0.2X and 2X depending on the specific experiment.

Reagents	Reactions number		
	1	10	100
Nile Red (1000X)	1 μL	10 μL	100 μL
Hoechst 33342 (1000X)	1 μL	10 μL	100 μL
Assay Buffer	998 μL	9.98 mL	99.8 mL
Total	1 mL	10 mL	100

2. Lipid droplet staining:

A. Staining of adherent cells

If adherent cells need to be detected with fluorescence spectrophotometer or flow cytometry, the cells can be harvested and digested first, and then suspended and operated according to the steps for suspended cells.

1) **Cell culture:** Seed cells in appropriate plates, petri dishes or flasks. Treat cells with the interested drug according to the experimental design.

2) **Positive control (optional):** For a positive control, the oleic acid can be added at a final concentration of 400 μM in culture medium for 24 h to induce the lipid droplets.

***Note:**

a) Oleic acid is partly toxic to cells, so the optimal concentration of oleic acid varies depending on the cell types.

b) This kit does not provide oleic acid, please purchase it on your own if necessary.

3) **Fixation (optional):** Remove the culture medium, and wash 2 times with PBS. Then add 4% formaldehyde to fix for 10-15 min at room temperature.

***Note:**

a) This step is required for fixed cell staining, not for live cell staining.

b) Because alcohols can solubilize lipids, aldehyde-based fixatives are recommended.

c) If immunofluorescence is also required and permeabilization is needed, do not use permeabilizers containing detergents such as Triton X-100 or Tween-20, and it is recommended to use a permeabilization solution containing Saponin or Digitonin that does not dissolve cell membranes, but this may still affect the morphology of the lipid droplets.

4) **Staining:** Wash 1-2 times with PBS before staining. Subsequently, incubate with staining solution for 10-20 min at room temperature in the dark. For 6-well plates, at least 1 mL of staining solution per well is needed. Other plate sizes can be used by scaling as necessary, but make sure that the staining solution can fully cover the cells.

***Note:** The optimal time for incubation varies depending on the cell types.

5) **Washing:** Remove the staining solution and wash 1-2 times with PBS. After adding an appropriate amount of PBS or other suitable buffers, in situ detection can be performed with fluorescence microscopy. If a black 96-well plate is used, it can also be detected with a fluorescence microplate reader.

B. Staining of suspension cells

1) **Cell culture:** Seed cells in appropriate flasks. Treat cells with the interested drug according to the experimental design.

2) **Positive control (optional):** For a positive control, the oleic acid can be added at a final concentration of 400 μM in culture medium for 24 h to induce the lipid droplets.

***Note:**

a) Oleic acid is partly toxic to cells, so the optimal concentration of oleic acid varies depending on the cell types.

b) This kit does not provide oleic acid, please purchase it on your own if necessary.

3) **Fixation (optional):** Centrifuge at 1000 rpm for 5 minutes to remove the culture medium, and wash 2 times with PBS. Then resuspend cells in 4% formaldehyde to fix for 10-15 min at room temperature.

***Note:**

a) This step is required for fixed cell staining, not for live cell staining.

b) Because alcohols can solubilize lipids, aldehyde-based fixatives are recommended.

c) If immunofluorescence is also required and permeabilization is required, do not use permeabilizers containing detergents such as Triton X-100 or Tween-20, and it is recommended to use a permeabilization solution containing Saponin or Digitonin that does not dissolve cell membranes, but this may still affect the morphology of the lipid droplets.

4) **Staining:** Centrifuge at 1000 rpm for 5 minutes to remove the supernatant, and wash 1-2 times with PBS. Subsequently, resuspend cells in staining solution to give a cell density of 10^6 cells/mL. Incubate at room temperature in the dark for 10-20 min.

***Note:** The optimal time for incubation varies depending on the cell types.

5) **Washing:** Centrifuge at 1000 rpm for 5 minutes to remove the staining solution, and wash 1-2 times with PBS. Resuspend cells in an appropriate amount of PBS or other suitable buffers. Then use a fluorescence spectrophotometer, fluorescence microplate reader, or flow cytometer for detection. Alternatively, an appropriate amount of cell suspension can be taken to make a smear for detection with a fluorescence microscope.

3. **Detection:** Monitor the fluorescence of Nile Red at Ex/Em=485/535 nm or Ex/Em=552/635 nm. Or directly use FITC filter sets (green) or TRITC filter sets (red) for detection. Monitor the fluorescence of Hoechst 33342 (Ex/Em: 350/461 nm) with the DAPI filter sets.

***Note:**

a) When using a fluorescence microscope, it is recommended to minimize the intensity of the excitation light and shorten the exposure time to minimize fluorescence quenching.

b) When using flow cytometry, the concentration of the Nile Red probe may be lower than fluorescence microscopy needed because flow cytometry is more sensitive, and the Nile Red probe concentration can be adjusted according to the specific experiment. It is also recommended to use a sample containing only Assay Buffer and not stained with probes as the negative control.

c) When using a fluorescence plate reader, it is recommended to use a black 96-well plate for detection.

Note

1. Nile Red is easy to quench and needs to be protected from light when storing and using. At the same time, it

is recommended to aliquot appropriately to avoid repeated freezing and thawing.

2. Nile Red is easy to quench under excitation light. So, it is recommended to reduce the concentration of the probe, reduce the intensity of the microscope excitation light and shorten the imaging time while ensuring the fluorescence brightness.
3. Nile Red is not suitable for multicolor fluorescence staining due to its wide range of emission spectrum.
4. For your safety and health, please wear lab coats and gloves during the experiment.
5. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com