Green Fluorometric Lipid Droplet Assay Kit (BODIPY 493/503)

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.

Green Fluorometric Lipid Droplet Assay Kit (BODIPY 493/503) is a kit for the detection of intracellular lipid droplets using the green fluorescent probe BODIPY 493/503. BODIPY 493/503 is a fluorescent probe that can label neutral lipids. BODIPY 493/503 is cell-penetrating, which can be used for staining live and fixed cells. BODIPY 493/503 is virtually non-fluorescent in water or other polar solvents, and emits a strong green fluorescence once combined with neutral lipids such as triglycerides. The maximum excitation and emission wavelength of BODIPY 493/503 are 493 nm and 503 nm.

Oil Red O and Nile Red are 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; while Nile Red does not have the above problems, it is not suitable for multicolor fluorescence staining due to its wide range of emission spectrum. The BODIPY 493/503 probe used in this kit does not have these problems, and has the advantages of simple operation, strong specificity, and high sensitivity.

Components and Storage

Components	K2168-100 T	K2168-500 T
BODIPY 493/503 (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. BODIPY 493/503 (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 BODIPY 493/503 probes in the staining solution can be adjusted between 0.2X and 2X depending on the specific experiment.

Reagents	Reactions number		
	1	10	100
BODIPY 493/503 (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 the 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 BODIPY 493/503 (Ex/Em: 493/503 nm) with the FITC filter sets. 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 BODIPY 493/503 probe may be lower than fluorescence microscopy needed because flow cytometry is more sensitive, and the BODIPY 493/503 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. BODIPY 493/503 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. BODIPY 493/503 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. For your safety and health, please wear lab coats and gloves during the experiment.
4. 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