IMMUNOLOGICAL SCIENCES Product Data Sheet

IS5091 Lipid Droplets Fluorescence Assay Kit

Unit Size: 100 T

Product Description/Introduction

Lipid droplets are specialized lipid storage organelles in cells, which can regulate the storage and hydrolysis of neutral lipid in cells and provide dynamic energy balance for cells. In addition, lipid droplets are able to move along the cytoskeleton and interact with other organelles, playing an important role in the regulation of signal transduction. Abnormal accumulation of cellular lipid droplets occurs in several types of metabolic diseases, such as obesity, fatty liver, cardiovascular diseases, and pathological states.

Lipid Droplet Fluorescence Assay Kit is based on BODIPY 493/503, which is a lipophilic fluorescent probe capable of localizing to polar lipids, and can be used to calibrate the neutral lipid content of cells and tissues. This kit has been optimized and debugged by our R&D team, and has multiple advantages such as easy to use, stable performance and good reproducibility, etc. The higher the intracellular lipid content, the stronger the intensity of fluorescence generated, which can be detected by fluorescence microscope and flow cytometer. In addition, this kit additionally provides oleic acid as an inducer of triglyceride synthesis and storage, which can be used for positive control induction.

Storage and Shipping Conditions

Ship with dry ice; Store at -20℃ away from light for 6 months.

Product Content

Lipid droplet assay probe (2 mM)	100 μL
Lipid Droplet Assay Buffer	100 mL
Positive inducer (500 mM)	50 μL

Assay Protocol / Procedures

Note: Take 6-well plate adherent cells as an example, suspension cells need to be centrifuged every time the liquid is changed; Tissue samples directly refer to the staining and labeling steps, and the overall volume of reagents used is adjusted according to the situation; If necessary, the samples can be fixed in advance, but please avoid perforation of the samples.

1. Preparation of working solution for lipid droplet assay and working solution for lipid droplet induction:

1.1. Take appropriate amount of lipid droplet assay probe (2 mM) and lipid droplet assay buffer, mix thoroughly at a ratio of 1 : 500-2000, and prepare a working solution for lipid droplet assay;

1.2. Take an appropriate amount of positive inducer (500 mM) and use the cell culture medium of the corresponding test cells to dilute it to 400-1000 uM, and prepare a lipid droplet induction working solution for spare use (this concentration is a reference range, and can be adjusted appropriately according to the nature of the cells or other circumstances).

2. Positive control group and drug and other treatments (6-well plate adherent cells, for example, suspension cells each time the change of liquid, please perform centrifugation):

2.1. Cells are pre-planted in 6-well plates as needed (with or without crawler depending on assay and equipment);

2.2. Remove the original cell culture medium and wash twice with PBS or other buffers;

2.2.1. Experimental group: the cells to be tested are subjected to drug stimulation or other relevant treatments, and the desired incubation time is set by yourself;

2.2.2. Positive control group: 1 mL of lipid droplet induction working solution is added to the well plate, which is placed in an incubator for overnight incubation;

2.3. At the end of the incubation, turn to step 3 for stain labeling.

3. Lipid droplet staining marker:

3.1. Remove the cellogenic medium and wash 2-3 times with PBS or other buffers;

3.2. Remove the wash buffer, add 1 mL of Lipid Droplet Assay Working Solution and mix with gentle shaking;

3.3. Incubate for 15 min in a CO2 incubator protected from light;

3.4. After incubation, wash it twice with PBS or other buffers, and it can be further stained with nuclei and other operations or detected with the appropriate equipment according to the experimental needs;

4. Lipid droplet detection:

4.1. The sample after incubation and washed in 3.4 is analyzed using the appropriate method and instrumentation, labeled to show green fluorescence, EX/EM 493/503 nm, and some examples of instrumental detection are given below;

4.1.1. Directly or with anti-fluorescence quenching sealing solution, the film is sealed and then placed under a fluorescence microscope for detection and analysis.

4.1.2. After digestion and resuspension using tryptic digest (suspension cells are not used), cells are detected and analyzed using flow cytometry.

Note

1. Due to the different types and properties of cells, the concentration of positive inducers and probes and the incubation time can be adjusted appropriately according to the needs of the experiment.

2. Samples should avoid contact with organic solvents or other components that affect lipids to avoid interfering with the accuracy of the assay.

3. The fluorescent dyes are subject to quenching, and the process should be protected from light.

4. For your health and safety, please wear lab coat and gloves during operation.