Servicebio® DiO Cell Membrane Green Fluorescence Staining Kit

Cat. #：G1704

Product Information

Product Name	Cat. No	Spec.
DiO Cell Membrane Green Fluorescence Staining Kit	G1704	100-1000 T

Product Description/Introduction

DiO, also known as DiOC18(3), 3-octadecyl-2-[3-(3-octadecyl-2(3H)-benzoxazol-2-ylidene)-1-propen-1-yl]benzoxazole perchlorate, with a molecular weight of 881.72, is a class of lipophilic, long-chain dialkyldicarbocyanine dyes, fluorescent dyes, commonly used in the labeling of cell membranes and other fat-soluble biological structures. After entering the cell membrane, DiO can diffuse laterally and gradually stain the whole cell membrane.DiO fluorescence is very weak before entering the cell membrane, and the fluorescence intensity will be greatly enhanced when it binds to the cell, and it can emit green fluorescence after excitation and can be detected by standard FITC filters. The maximum excitation wavelength of DiO is 484nm, and the maximum emission wavelength is 501nm. According to the characteristics of DiO, it can stain living cells as well as fixed cells. In addition. DiO probes generally do not affect the viability of cells, so forward or reverse labeled cells or some substances (exosomes) can be used as tracer detection.

The DiO Cell Membrane Green Fluorescence Staining Kit contains DiO fluorescence probe and optimized staining buffer, which can make cell membrane staining faster, fluorescence more bright and stable.

Storage and Shipping Conditions

Ship with dry ice; store at -20°C in the dark, valid for 6 months.

Product Contents

Component Number	Component	G1704
G1704-1	DiO cell membrane green fluorescent probe	400 μL
G1704-2	Staining buffer	100 mL
Manual		One copy

Assay Protocol / Procedures

1. Preparation of DiO staining working solution:

1.1. Mix and dilute DiO Cell Membrane Green Fluorescent Probe with Staining Buffer at a ratio of 1:250 to prepare DiO Staining Working Solution (ready to use); note that the DiO probe dilution ratio can be adjusted at 1:250-1:500 according to the specific situation in order to obtain the best staining effect.

2. Staining of live suspension cells

2.1. Centrifuge the suspended cells at 500-1,000×g at room temperature for 3-5 min, remove the cell supernatant;

2.2. Add appropriate amount of Dio staining working solution to resuspend cells to a final cell density at 1-2×10^6 cells/mL.

2.3. Incubate at 37℃ in the dark for 5-20 min. The optimal incubation time varies for different cells. Start with 5 min and optimize the incubation time based on the final staining result.

Wuhan Servicebio Technology Co.,Ltd.
Add: 5th Floor,22 building, Biopark, Gaoxin Second Road 388, East Lake High-Tech Developing Zone,Wuhan,China 430000
Tel: 027-5111-3188 Website: www.servicebio.com

2.4. At the end of incubation, centrifuge at 500-1000 g for 3-5 min at room temperature and aspirate the DiO staining working solution;

2.5. Cells were resuspended with pre-warmed PBS or cell culture medium at 37°C and centrifuged at 500-1000 g for 3-5 min to remove the supernatant;

2.6. Repeat step 2.5.

2.7. Detected by flow cytometry directly or by fluorescence microscopy after transfering cells to a multi-well plate, cell culture dish or cell climbing slide. Dio has an excitation maximum at 484 nm and an emission maximum at 501 nm.

3. Staining of live adherent live cells (6-well plate as an example):

3.1. Seed adherent cells in 6-well plate at a certain density.

3.2. Remove the culture medium and wash cells twice with PBS (Recommend G4202). Add 1 mL of Dio staining working solution (other size well plates, adjusted as appropriate to ensure that the dye covers the cells);

3.3. Incubate at 37℃ in the dark for 5-20min. The optimal incubation time varies for different cells. Start with 5 min and optimize the incubation time based on the final staining result.

3.4. Aspirate the cell membrane staining working solution and wash cells 1-2 times with preheated PBS or cell medium.

3.5. Add pre-warmed cell culture medium or cell medium at 37℃ and detect cells by fluorescence microscopy. Dio has an excitation maximum at 484 nm and an emission maximum at 501 nm.

4. Staining of fixed adherent cells:

4.1. Sample preprocessing:

For cells: Remove the cell medium and wash 1-2 times with PBS. Add 4% paraformaldehyde fix solution (Recommend G1101) for 10 min at room temperature. Remove fix solution and wash 2-3 times with PBS;

4.2. Permeabilization: Add 0.1-0.5% Triton-100 (prepared with PBS) and permeated for 10 min at room temperature. Remove permeabilization solution and wash 2-3 times with PBS.

4.3. (Optional, immunofluorescent labeling) Incubate with antibodies according to immunostaining protocol or incubate with other dyes.

Note: Blocking solution, antibody diluent, and wash solution for immunostaining should not contain detergents.

4.4. Add an appropriate amount of DiO staining working solution to cover the cells, incubate at 37°C away from light for 5-20 min, and aspirate the DiO staining working solution. It is recommended that the staining time be adjusted to the specific cell sample to obtain optimal staining results;

4.5. Cells were washed 2-3 times with PBS and then placed under a fluorescence microscope for observation (cells need to be covered with appropriate amount of PBS).DiO has a maximum excitation wavelength of 484nm and a maximum emission wavelength of 501nm.

5. Fluorescent labeling of exosomes:

5.1. Resuspended exosome precipitation with an appropriate amount of DiO staining working solution.

5.2. Incubate at 37℃ for 30 min in the dark.

5.3. (optional) Dilute the sample with 10-fold volume PBS.

5.4. Extracted exosomes again according to the previous extraction protocol to remove excess dyes.

5.5. Collect exosomes precipitation and resuspended in PBS to obtain DIO-labeled exosomes. It can be used for subsequent experiments, such as cellular uptake.

Note

Wuhan Servicebio Technology Co.,Ltd.
Add: 5th Floor,22 building, Biopark, Gaoxin Second Road 388, East Lake High-Tech Developing Zone,Wuhan,China 430000
Tel: 027-5111-3188 Website: www.servicebio.com

1. All fluorescent dyes have quenching problems, please protect from light during the operation to slow down fluorescence quenching.
2. Due to the probe is lipophilic, please avoid using reagents containing glycerin or other organic matter.
3. If fixation is required, we recommend to fix in 4% paraformaldehyde. Other inappropriate fix solutions will lead to high fluorescence background.
4. The optimum dilution ratio and incubation time of the probe should be adjusted according to the actual situation due to the different sensitivity between cells and experimental requirement.
5. For your safty and health, please wear safety glasses, gloves, or protective clothing.

For Research Use Only!

Ver. No.: V2.0-202308

Wuhan Servicebio Technology Co.,Ltd.
Add: 5th Floor,22 building, Biopark, Gaoxin Second Road 388, East Lake High-Tech Developing Zone,Wuhan,China 430000
Tel: 027-5111-3188 Website: www.servicebio.com