DiO Membrane Staining Kit

User Manual

Catalog # CRG1066

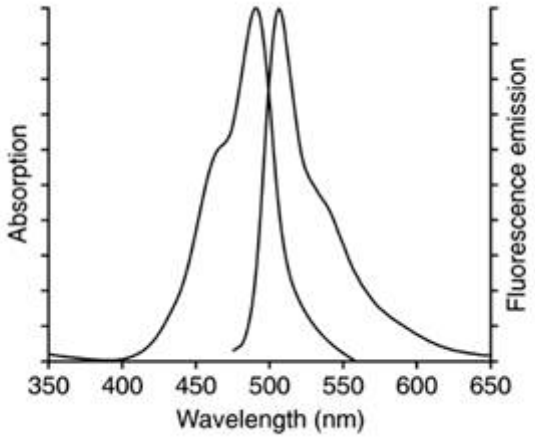
Highly green fluorescent membrane probe for labeling live and fixed cells

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

DiO (3,3'-dioctadecyloxacarbocyanine perchlorate) (CAS# 34215-57-1), has the molecular formula of $C_{53}H_{85}ClN_2O_6$ with the molecular weight of 881.72. DiO is one of the most commonly used fluorescent probes for cell membrane staining. It is a lipophilic carbocyanine dye with a long lipophilic hydrocarbon chain, enabling its lateral diffusion in cell membrane.

Free DiO fluoresces weakly. However, when entering the cell membrane, it can emit strong green fluorescence at 501nm when excited at 484nm. Please refer to Figure for the excitation and emission spectra of DiO after binding to phosphate bilayer of cell membrane.



DiO is widely used as a tracer or long-term tracer for positive or retrograde, live or fixed cells or tissues. It has low toxicity and usually does not affect cell viability. However, it has a lower fluorescence intensity than DiI for cell membrane staining, and is sometimes less effective for staining some particular fixed tissues.

In addition to the fluorescent labeling of cell membranes, DiO can also be used to detect cell fusion and adhesion, cell migration during development or transplantation, lipid diffusion across cell membranes by FRAP (Fluorescence Recovery After Photobleaching), cytotoxicity and lipoproteins labeling etc.

II. KIT COMPONENTS

Component	50 Assays	300 Assays	Storage
DiO Probe (100X)	50 µl x 1	300 µl x 1	4 °C
Dilution Buffer	5 ml x 1	30 ml x 1	4 °C
Technical Manual	1 Manual	1 Manual	

III. STORAGE AND STABILITY

Shipped at 4°C. Store at -20°C and protect from light for 12 months.

IV. WORKING SOLUTION PREPARATION

Working Solution: Dilute 1 µl DiO Probe (100X) in 100 µl Dilution Buffer.

Note: The dilution ratio can be adjusted appropriately according to the experimental effect.

Ex/Em = 484/501 nm

V. ASSAY PROCEDURE

1. For adherent cells staining

(1) Adherent cells were cultured on a sterile cover slide.

(2) Remove the cover glass from the medium, absorb excess liquid but keep the surface moist.

(3) Add 100 µL of Working Solution to one corner of the cover glass and gently shake to evenly cover all cells.

(4) Cells were incubated at 37 °C for 2-20 mins. The reaction time can be optimized to obtain uniform labeling effect.

(5) Discard Working Solution, wash the glass with PBS for 2 to 3 times.

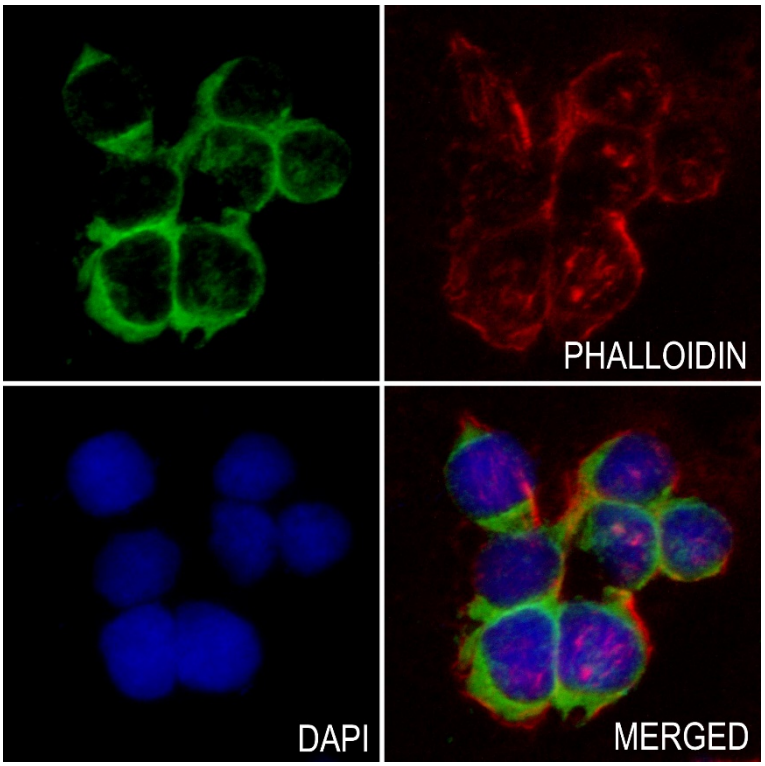
2. For suspension cells staining

(1) Adding an appropriate volume of Working Solution to re-suspension cells, the density of the cells is 1×10^6 /mL.

(2) The cells were incubated at 37°C for 2-20 min. The reaction time can be optimized to obtain uniform labeling effect.

(3) After incubation, centrifuge at 1000-1500 rpm for 5 mins. Discard the supernatant and slowly add the growth medium again to resuspend the cells.

(4) Repeat step (3) more than twice.



Immunofluorescent analysis of Dio staining in MCF cells. Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with the primary antibody in 3% BSA-PBS and incubated overnight at 4 °C in a hidified chamber. Cells were washed with PBST and incubated with Dio (green) at room temperature in the dark. Phalloidin - AF594 was used to stain Actin filaments (red). DAPI was used to stain the cell nuclei (blue).

VI. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VII. NOTES