DiD Membrane Staining Kit

User Manual

Catalog # CRG1068

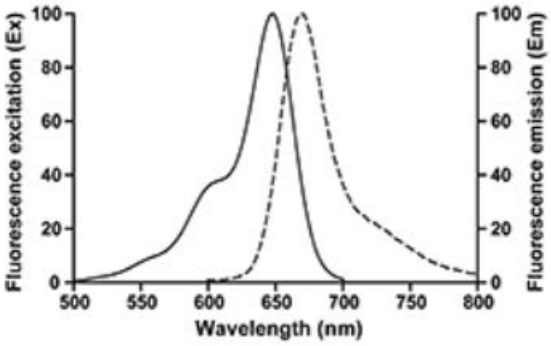
Highly red fluorescent membrane probe for labeling live and fixed cells

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

I. INTRODUCTION

DiD (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindodicarbocyanine,4-Chlorobenzenesulfonate Salt) is a far-red plasma membrane fluorescent probe with the molecular formula of C67H103ClN2O3S and the molecular weight of 1052.08. DiD is an analog of DiI, but has longer excitation and emission wavelength. DiD exhibits far-infrared fluorescence, which is more suitable for multiplex fluorescence staining of samples. DiD is a carbocyanine dye with a very long lipophilic hydrocarbon chain, enabling its lateral diffusion inside cell membrane.

Free DiD fluoresces weakly. However, when entering the cell membrane, it can be excited by helium-neon (He-Ne) laser and exhibits strong far-red fluorescence. It has an emission maximum at 665nm when excited at 644nm.



In addition to the simplest fluorescent labeling of cell membranes, DiD 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 lipoprotein labeling.

Live cells or tissues can be directly stained by DiD, which takes 5-20 minutes usually. If sample fixation is required before staining, we recommend using 4% paraformaldehyde formulated in PBS for fixation. The use of other inappropriate fix solutions may result in high fluorescence background.

II. KIT COMPONENTS

Component	50 Assays	300 Assays	Storage
DiD 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 DiD Probe (100X) in 100 µl Dilution Buffer.

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

Ex/Em = 644/663 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.

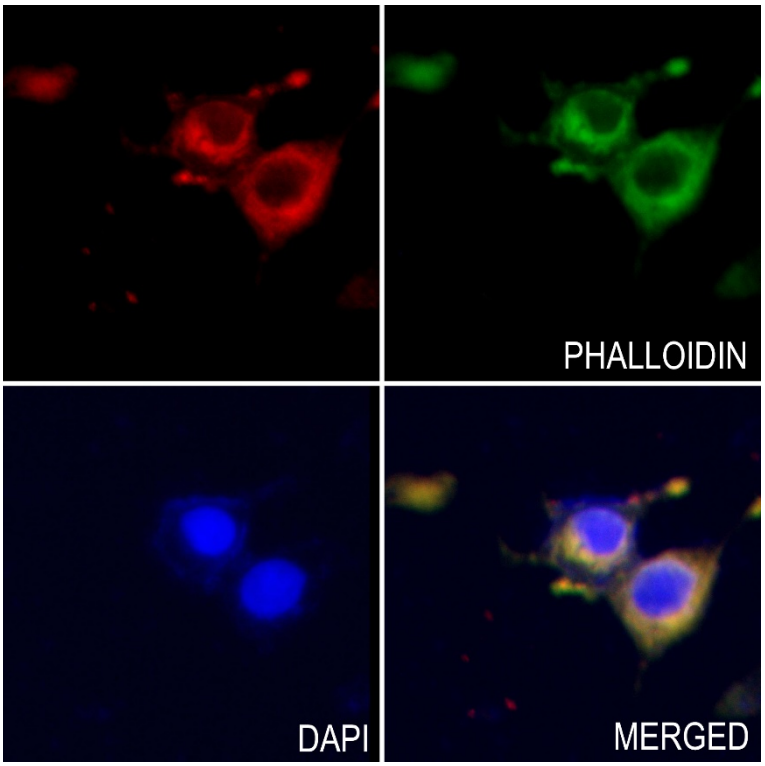
2. For suspension cells staining

(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 DiD staining in PC3 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 DiD (red) at room temperature in the dark. Phalloidin - AF488 was used to stain Actin filaments (green). 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