Cell Membrane Staining Kit (DiD)

Introduction

DiD, also called $DiDC_{18}(5)$, is a red fluorescent probe that is widely used as a lipophilic tracker in living and fixed tissues and cells. DiD can insert into the membrane, and diffuse rapidly, staining the entire membrane surface red. DiD usually does not affect cell viability and development, so it is commonly used in cell tracking, such as anterograde and retrograde neuronal tracking. DiD is excited by the 633 nm He-Ne laser, and has longer excitation and emission wavelengths than DiI, providing an alternative for labeling cells and tissues that have significant intrinsic fluorescence.

DiD staining can be used in conjunction with immunofluorescence. It is recommended to use formaldehyde (PFA) for fixation after DiD staining. Meanwhile, if permeabilization is required, it is suggested to use Triton X-100 or digitonin. But permeabilization may affect the localization of DiD in the cell membrane. In addition, DiD has many other applications including detecting cell-cell fusion and adhesion, tracking cell migration, and labeling lipoproteins.

This kit is an optimized DiD staining kit that not only provides ready-to-use DiD dye, but also provides a Staining Enhancer for better staining results and lower staining background. For 96-well plates, the kit can be used 1000 times using 100 μL of staining solution per well.

Composition and storage conditions

Components \ Size	1000 Assays	Storage
DiD (200×)	0.5 mL	-20°C away from light
Staining Enhancer (200×)	0.5 mL	-20°C
Staining Buffer	100 mL	-20°C
Shipping: Blue ice	Shelf life: 1 year	

Experimental manipulation

1. Preparation of the working solution

1) Refer to the following table to prepare the working solution, the working solution is unstable and needs to be prepared freshly. DiD (200×) and Staining Enhancer (200×) should be thoroughly mixed before use. For 96-well plates, 100 μL of staining solution is required per well. For 6-well plates, 1 mL of staining

solution is required per well.

Number of samples	10	100	1000
DiD (200×)	5 μL	50 μL	500 μL
Staining Enhancer (200×)	5 μL	50 μL	500 μL
Staining Buffer	990 μL	9.90 mL	99 mL
Total	1 mL	10 mL	100 mL

***Note:** The working solution concentration can be adjusted and optimized according to the cells and experimental system.

2. Staining of suspension cells

1) Harvest cells and centrifuge at 1000 rpm for 5 min, remove the supernatant. Suspend the cell pellet in a suitable working solution at a density of 1-2 x 10^6 cells/mL.

2) Incubate at 37°C away from light for 2-20 min. The optimal incubation time varies depending on the cell type.

3) Centrifuge at 1000 rpm for 5 min, remove the supernatant.

4) Gently resuspend the cell pellet in a pre-warmed growth medium, and wash 2 times.

5) Gently resuspend the cell pellet in a suitable buffer (serum-free medium or PBS), then watch by a microscope or flow cytometry (Ex/Em= 644/665 nm). DiD can be watched directly with a Cy5 filter.

3. Staining of adherent cells

1) Grow adherent cells on cell dishes, cell plates or sterile glass coverslip.

2) Remove the growth medium and wash cells twice with PBS.

3) Add the working solution to cover the cells. For 96-well plates, 100 μL of staining solution is required per well. For 6-well plates, 1 mL of staining solution is required per well. Incubate at 37°C away from light for 2-20 min. The optimal incubation time varies depending on the cell type.

4) Remove the working solution and wash the cells twice with PBS.

5) Gently add a suitable buffer (serum-free medium or PBS) to cover the cells, then watch by a microscope (Ex/Em= 644/665 nm). DiD can be watched directly with a Cy5 filter.

4. Fix after staining

1) If further immunofluorescence experiments are required after staining, fix cells with 4% PFA.

2) If permeabilization is also required, permeabilization with 0.1% Triton X-100 or digitonin is recommended. However, permeabilization is likely to affect the staining of cell membranes by DiD and increase the staining background.

3) In addition, detergents may dissolve lipids on the cell membrane and affect the localization of the cell membrane of DiD, so blocking solutions, antibody diluents, and washes should not contain detergents. It

is recommended to mount directly with PBS. Do not use mounting media containing glycerin or other organic matter, as this will affect the staining effect.

5. **Staining after fixation**

1) Fix the cells with 4% PFA.

2) If permeabilization is also required, permeabilization with 0.1% Triton X-100 or digitonin is recommended. However, permeabilization is likely to affect the staining of cell membranes by DiD and increase the staining background.

3) Perform the immunofluorescence staining as usual. Detergents may dissolve lipids on the cell membrane and affect the membrane localization of DiD, so blocking solutions, antibody diluents, and washes should not contain detergents.

4) Add the appropriate working solution to cover the cells. For 96-well plates, 100 µL of staining solution is required per well. For 6-well plates, 1 mL of staining solution is required per well. Incubate at 37°C away from light for 2-20 min. The optimal incubation time varies depending on the cell type.

5) Remove the working solution and wash the cells twice with PBS.

6) Gently add a suitable buffer (serum-free medium or PBS) to cover the cells, then watch by a microscope (Ex/Em= 644/665 nm). DiD can be watched directly with a Cy5 filter.

Note

1. Fluorescent probes are easy to quench, please protect them from light when using.
2. If the labeling time is too long or cells are cultured after staining, the probe may also enter the cell to stain other organelles.
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.

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