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.

Components and Storage

Components	B8805-5 mg	B8805-10 mg
DiD	5 mg	10 mg
The product should be stored at -20°C away from light and moisture. Stable for 1 year. The stock solution should be stored at -20°C away from light. Stable for 6 months.		

Protocol

1. Preparation of stock solution and working solution

1) Prepare a stock solution of DiD in dimethylsulfoxide (DMSO), or ethanol at 1 mM.

***Note:** The stock solution should be stored at -20°C and avoid repeated freeze/thaw cycles. The stock solution is stable for 6 months at -20°C.

2) Dilute the stock solution into a suitable buffer (serum-free medium, HBSS or PBS) to make a 1-30 μM working solution. The commonly used concentration of the working solution is 5-10 μM. The working solution is unstable, so it is better to dilute the stock solution when using it.

***Note:** The optimal concentration of the working solution varies depending on the cell type and experimental condition.

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 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. The maximum excitation wavelength of DiD is 644 nm and the maximum emission wavelength is 665 nm. DiD can be watched directly with a Cy5 filter.

3. **Staining of adherent cells**

    1) Grow adherent cells on sterile glass coverslip.

    2) Remove the coverslip from growth medium and gently remove excess medium.

    3) Add 100 μL working solution onto the coverslip, and gently shake to cover the cells. 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 coverslip with pre-warmed growth medium two times, 5-10 min/per time.

***Note:** During the process, pay attention to keeping the surface of the coverslip moist and prevent drying.

    5) Cells can be imaged in a suitable buffer (serum-free medium or PBS) with a microscope. The maximum excitation wavelength of DiD is 644 nm and the maximum emission wavelength is 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 fixed cells or tissues, formaldehyde (PFA) is recommended use for fixation. Meanwhile, if permeabilization is required, it is suggested to use Triton X-100 or digitonin, but permeability may affect the localization of DiI in the cell membrane. In addition, detergents may dissolve lipids on cell membranes and affect the cell membrane localization of DiI, so do not use detergents in the buffer used for blocking, antibody dilutions, and washing. When sealing, it is recommended to directly use PBS for sealing. Do not use mounting agents containing glycerol or other organic solvents, as this will affect the staining effect.

4. For your safety and health, please wear lab coats and gloves during the experiment.

5. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com