DiR

Introduction

DiR, also called $DiIC_{18}(7)$, is a deep-red fluorescent probe that is widely used as a lipophilic tracker in living and fixed tissues and cells. DiR can insert into the membrane, and diffuse rapidly, staining the entire membrane surface red. DiR usually does not affect cell viability and development, so it is commonly used in cell tracking, such as anterograde and retrograde neuronal tracking. DiR has remained viable for up to four weeks in culture and up to one year in vivo.

DiR is useful for in vivo imaging due to the effective transmission of infrared light through cells and tissues and low autofluorescence in the infrared range. In addition, DiR has many other applications including detecting cell-cell fusion and adhesion, tracking cell migration, and labeling lipoproteins.

Components and Storage

Components	B8806-5 mg	B8806-10 mg
DiR	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 DiR 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). The maximum excitation wavelength of DiR is 748 nm and the maximum emission wavelength is 780 nm. The fluorescence emission of DiR is invisible to human eyes and should be detected by a CCD camera or other infrared-sensitive detector.

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). The maximum excitation wavelength of DiR is 748 nm and the maximum emission wavelength is 780 nm. The fluorescence emission of DiR is invisible to human eyes and should be detected by a CCD camera or other infrared-sensitive detector.

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.

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