

DYE FILLING TO STAIN AMPHID AND PHASMID NEURONS

Reagents:

M9 media

DiO (Molecular Probes) – 2mg/ml in dimethyl formamide. Store at –20°C in foil

Procedure:

1. Dilute DiO stock solution 1:200 in M9. Some dye will precipitate but don't worry about it.
2. Dispense 150µl stain into the well of a microtiter plate; use a worm pick to transfer a few worms into the dye. Incubate 2-3 hours at room temperature.
3. Transfer the worms to a fresh plate and let them crawl on a bacterial lawn for about 1 hour to destain OR wash the worms 3 times in M9.
4. Put the worms on agar pads with sodium azide and visualize by fluorescence using the appropriate filters.

Comments:

DiO fluoresces green (use FITC filter). If red fluorescence is desired, substitute DiI and Texas red filters. DiI may stain a slightly different set of cells.

References:

Beth Sawin, Dartmouth University

Lipophilic Tracers – DiI, DiO, DiD, DiA, and DiR. Molecular Probes Product Information