

# Synthesis of Caged Fluorescein Dextran

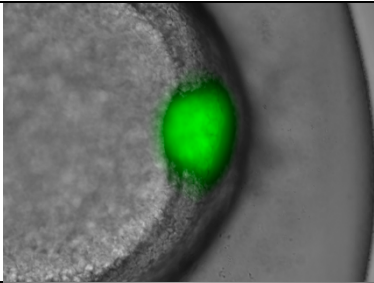
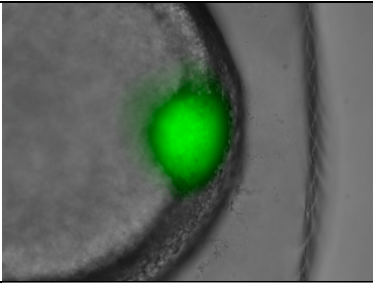
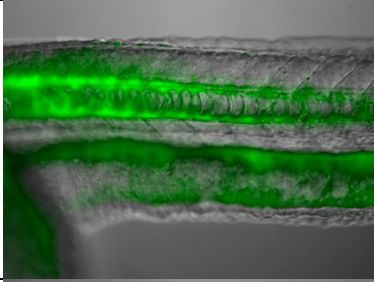
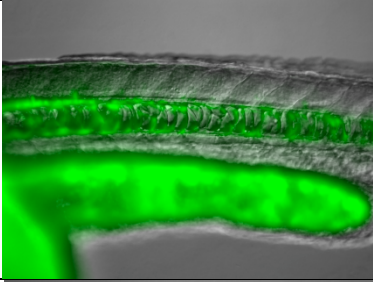
## Reagents and Materials

CMNB-caged fluorescein SE (Invitrogen C20050)  
Aminodextran, 10 kDa (Invitrogen D1860)  
Zeba Desalt Spin Column (Pierce 89889)  
0.1 M  $\text{Na}_2\text{B}_4\text{O}_7$ , pH 8.5  
15-mL polypropylene conical tubes (such as Falcon 352097)  
1.5-mL microcentrifuge tubes  
Vortexing mixer  
Centrifuge capable of spinning 15-mL tubes at 1000 x *g*  
Speed-vac

## Procedure

- 1) Measure out 3.5 - 4 mg of aminodextran and add into the Invitrogen-supplied tinted tube containing 1 mg of CMNB-caged fluorescein SE. In our hands this ratio gives an average loading of ~ 2.5 dye molecules per dextran.
- 2) Add 500  $\mu\text{L}$  of 0.1 M  $\text{Na}_2\text{B}_4\text{O}_7$  (sodium borate) buffer to the tube.
- 3) Cap and vortex for 30 seconds to dissolve the aminodextran and caged fluorescein.
- 4) Let react overnight on a vortexing mixer.
- 5) Twist off the bottom closure on a Zeba spin column and loosen the cap. Mark a dot on the column with a felt-tip pen. Place column in a 15-mL conical tube.
- 6) Centrifuge at 1000 x *g* for 2 min with the dot facing the center of the rotor. Use column immediately after compacting.
- 7) Place compacted column in a new 15-mL conical tube.
- 8) Pool reaction mixture and transfer to the center of the compacted column resin bed. Replace cap.
- 9) Centrifuge at 1000 x *g* for 2 min with the dot facing the center of the rotor. After the spin, the eluent and the top of the column will be roughly the same shade of yellow in color.
- 10) Transfer the yellow eluent (~350  $\mu\text{L}$ ) to a 1.5-mL microcentrifuge tube.
- 11) Lyophilize to dryness in a speedvac. Cover the speedvac with foil.
- 12) Re-suspend the resulting ~ 2-3 mg of pale yellow foam in water to a final concentration of 1% w/v.
- 13) Store this solution at -20 °C in foil-covered tubes. It is a good idea to aliquot the dextran solution to prevent repetitive freeze-thawing.
- 14) For injection, dilute 10-fold in injection solution for 2-nL/embryo injections. As with morpholinos, caged fluorescein dextran can be injected into the animal cell or the yolk.

## Results

	Discontinued Invitrogen caged fluorescein dextran (D-3310)	Above-described reagent
Local irradiation (dot) at 6 hpf in the shield.		
Result of above photoactivation at 24 hpf.		
Local irradiation (X) at 24 hpf.	