

Staining using lipophilic fluorescent dyes

Ito, 20.8.95

Dil Molecular probes cat.#d-282, 55\$ for 100 mg

red can be seen with rhodamin optics

DiO Molecular probes cat.#d-275, 55\$ for 100 mg

yellow can be seen with fluorescein optics

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1. dissolve DiO or Dil in EtOH or DMF (N,N,dimethylformamid)
DMF works better
conc. 0.2-0.3% in DMF for injection into tracts.
store 4°C
2. pull a thinned walled, fiber-filled† capillary† tube† to† 1µm,† blunt† ended electrode.
3. backfill the tip of the electrode by dipping the other end in the dye.
4. set the electrode to the pressure injector.
default parameters: 10-100 msec pulses at 60-80 psi
for tightly packed nerve tissue
lower pressure for looser tissue
5. fix the tissue with 2% paraformaldehyde for 15-20 min.
6. inject
7. let the dye to spread for 2-5 hrs.
8. before viewing, add final 0.05% vitamin C to the buffer
vitamin C retards photobleaching

<for making eternal preparations>

9. add DAB to the buffer
10. light with UV until the dye completely bleach
11. light for more than a few min. after bleaching
12. ODAB precipitate around the tracts