**Retrograde labelings of peripheral nerves by carbocyanine dye (DiI, DiO, DiD) (in vivo method)**

Procedure

Injections

1. Anesthetize Lewis rats (200–400 g) with 4% isoflurane and place them under a dissection microscope.

2. Inject 100–200 µl 0. 5-1.0% dye solutions into peripheral tissue (eg. Subcutaneous injection into whisker pads using a 25-µl Hamilton syringe.

3. Two weeks survival.

Perfusion, Sectioning

4. Under deep urethan or pentobarbital anesthesia, transcardially perfuse the rat with ice-cold saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Postfix overnight with the same fixative.

5. Prepare 50 µm -thick coronal brain stem sections with a vibrating blade microtome and collect the sections into 0.1 M PB.

Mounting, cover-slipping

6. Mount on gelatin-coated glass slides and cover-slip with an aqueous mounting medium (eg. PermaFluor). Avoid mounting medium with glycerol like Prolong Gold, Vectashield Hard Set, Aqua-Poly Mount etc.

Observation

7. Observe with epifluorescence or confocal microscopy.

**Ex Em**

DiO 484 501

DiI 549 565

DiD 644 665

**Solution**

**Stock solution**

DiO 100 mg in N,N-dimethylformamide (DMF) 1 ml (10%)

DiI 100 mg in DMF 1 ml (10%)

DiD 25 mg in DMF 0.25 ml (10%)

**Working solution**

DiO 0.5% in DMF

DiI 1% in EtOH

DiD 1% in EtOH

**Reagents**

DiO (D275, Thermo Fisher Scientific)

DiI (D282; Thermo Fisher Scientific)

DiD (D307, Thermo Fisher Scientific)

N,N-dimethylformamide (045-02916; Wako)

PermaFluor Aqueous Mounting Medium (TA-030-FM; Lab Vision Corporation)

**References**

Invitorogen Manual MP00282, Lipophilic Tracers-DiI, DiO, DiD, DiA, and DiR