SOP 73 - Kleinfeld Laboratory - 14 June 2017

Plasticizing of stained brain

From Shawn Mikula and Winfried Denk (*High-resolution whole-brain staining for electron microscopic circuit reconstruction, 2015, Nat Meth **12**:451-456*) and Shawn's update.

Transfer steps

All steps take place in a 50-ml centrifuge tube.
All solution volumes are 50 ml.

100 % water		96 hrs	4 °C
Transfer the brain to a new 50-ml centrifuge tube.			
10 % ethanol	90 % water	18–24 hrs	4 °C
25 % ethanol	75 % water	18–24 hrs	4 °C
50 % ethanol	50 % water	18–24 hrs	4 °C
75 % ethanol	25 % water	18–24 hrs	4 °C
100 % ethanol		18–24 hrs	4 °C
100 % propylene oxide		18–24 hrs	4 °C
75 % propylene oxide	25 % epoxy	18–24 hrs	4 °C
50 % propylene oxide	50 % epoxy	18–24 hrs	4 °C
25 % propylene oxide	75% epoxy	18–24 hrs	4 °C
100 % epoxy		18–24 hrs	4 °C
Transfer brain to a silicone mold (7 mm × 10 mm × 18 mm ID for whole brain)			
Cure		48 hrs	60 °C

Total procedure time is 12 to 16 days.

Epoxy formulation (modification from Spurr[1])**:**

10 g	Vinylcyclohexene dioxide (VCHD or ERL-4206)
20 g	Nadic methyl anhydride (NMA; Electron Microscopy Sciences)
0.45 g	Dimethylaminoethanol (DMAE; Serva).

Notes: Compared with the original formulation, the acetic anhydride, nonenyl succinic anhydride (NSA), was replaced by NMA, which among the anhydrides commonly used as hardeners (NMA, NSA, and dodecenyl succinic anhydride (DDSA)) yields the hardest blocks while also penetrating well.

Compared with the original formulation, they used only VCHD rather than a mixture of VCHD and diglycidyl ether of polypropylene glycol (D.E.R. 736) because the latter reduces block hardness.

[1] AR Spurr. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J Ultrastruct Res,* **26**:31–43.