**Periodate-Lysine-Paraformaldehyde (PLP) Fixation**

1. Perfusion fix with 5mL PLP.
2. Remove tissues and place in PLP to fix for 3h at room temp (or overnight at 4oC).
3. Wash tissues in PBS, and equilibrate at 4oC in 7.5% Sucrose solution overnight, followed by 15% Sucrose solution for 4 hours, and 30% Sucrose solution for 2 hours.
4. Place tissues into 3 successive wells of OCT. Equilibrate for 5-10 minutes in each well to allow OCT to permeate tissue.
5. Freeze tissue in OCT blocks as usual.

Solutions:

1. Lysine-Phosphate Buffer: 0.375M Lysine (L-Lysine HCl, Sigma Cat# L5626) in 0.2M NaPO4, pH 7.4. Store at 4oC.
2. 4% Paraformaldehyde: 10mL 16% PFA in 30mL 0.1M NaPO4. Make fresh, or store at -20oC.
3. PLP: 2 parts Lysine-phosphate buffer, 5 parts 4% PFA, 3 parts dH20. For each 100mL of solution, add 0.2g NaIO4 (sodium periodate). This buffer must be prepared fresh each time.
4. 30% Sucrose in 0.05M NaPO4, pH 7.4
5. 15% Sucrose in 0.05M NaPO4, pH 7.4
6. 7.5% Sucrose in 0.05M NaPO4, pH 7.4

Note: To properly prepare NaPO4, see the recipe for Sorensen’s Phosphate Buffer

**Sorensen’s Phosphate Buffer 0.2M NaPO4:**

1. **Solution X:** 35.61g **Na2HPO4** in 1L H2O
2. **Solution Y:** 27.6g **NaH2PO4** in 1L H2O

For 50mL of Sorensen’s buffer: 40.5mL of solution X + 9.5mL of solution Y