

HUMAN PANCREAS PROCESSING FOR FIXED CRYOSECTIONS (OCT)

I. Reagents

- **1X PBS/10 mM PBS, 1L:** 1.44 g Na₂HPO₄ (dibasic), 0.2 g KH₂PO₄ (monobasic), 8.0 g NaCl, 2.0 g KCl; pH 7.5. *Prepare from scratch or use 1X PBS without Ca/Mg, Invitrogen, 14190-144.*
- **100 mM PBS, 1L:** 12.07 g Na₂HPO₄ (dibasic), 2.04 g KH₂PO₄ (monobasic), 8.0 g NaCl, 2.0 g KCl; pH 7.5. Prepare fresh and keep at 4°C. Based on Electron Microscopy Sciences catalog, 100 mM PBS filtered through a 0.22 µm filter has a shelf life of 1 month at 4°C.
- **16% Paraformaldehyde (PFA):** Electron Microscopy Sciences, 15710. Right before fixation, prepare 4% paraformaldehyde solution. Open the vial containing 10 mL of 16% paraformaldehyde stock, transfer contents of vial into a 50-mL Falcon tube, add 30 mL of 100 mM PBS, mix and place the tube on ice.

Note: *Prepare as many tubes as many tissue slices will be procured considering approximately 1:50 tissue to fixative ratio.*

- **Sucrose:** Fisher Scientific, BP220-1. Prepare 30% sucrose solution: 15 g sucrose + 35 mL 10 mM PBS in a 50-mL Falcon tube. Place the tube on a rocker to dissolve sucrose and then keep at 4°C. Alternatively, 30% sucrose can be prepared in a larger quantity, filtered through a 0.22 µm and stored at 4°C for 1 month.

Note: *Consider approximately 1:50 tissue to sucrose ratio.*

II. Procedure

1. Collect 2-3-mm thick cross-sectional slices from the Head, Body, and Tail (see the pancreas mapping).
 2. Cut each slice into four quadrants (A, B, C, D) in a clockwise manner.
- Note:** *For donors < 1 year of age keep the entire slice intact.*
3. Transfer the tissue into a 50-mL Falcon tube containing 40 mL of freshly prepared fixative (4.0 % paraformaldehyde/100 mM PBS) and fix for 3 hours on ice under mild agitation using an adjustable tilt rocker (LabNet).
 4. Wash the tissue four times in 40 mL of 100 mM PBS on ice over the period of 2-3 hours under mild agitation using an adjustable tilt rocker. Blot the tube with paper towel before adding the fresh washing solution.
 5. Equilibrate the tissue in 40 mL of 30% sucrose/10 mM PBS at 4°C overnight. Tissue will settle to bottom of the tube.

Note: *If the pancreas is not cleaned sufficiently from fat, it will never drop to bottom.*

6. Prepare a cryomold (VWR, 25608-916) and fill it half way with an OCT compound (VWR, 25608-930). Pour contents of the tube with the pancreas into a 10-cm Petri dish. Pick the pancreas with a pair of fine forceps and blot it with Kimwipes to remove an excess of sucrose. Place the tissue into the OCT-containing cryomold. Using forceps, push the tissue lightly to bottom of the

cryomold. Add more OCT to fill completely the cryomold. Freeze the tissue on a dry ice block or by placing in a -80°C freezer.