

TISSUE RUN UP PROTOCOL

1. Place perfusion fixed tissue into 2.5% glutaraldehyde in 1x PBS (pH 7.4) for 120+ minutes.
 2. Cut up tissue to the proper size keeping in mind any orientation needed in the final block.
 3. Wash in 3 changes 1x PBS for 15 minutes each.
 4. Post fix specimens in 1% Osmium tetroxide overnight.
 5. Wash in 3 changes 1x PBS for 15 minutes each.
 6. Dehydrate in graded series of alcohol (in PBS) for 15 minutes each:
 - a. 30% ethanol
 - b. 50% ethanol
 - c. 70% ethanol
 - d. 90% ethanol (in H₂O)
 - e. 100% ethanol x 3
 7. Dehydrate further in two 10 minute changes of propylene oxide. *
 8. Infiltrate with a 1:1 mix of propylene oxide and epon overnight.
 9. Infiltrate with pure epon overnight at 4° C or 1 hour at room temperature.
 10. Continue infiltration with three 1 hour changes of epon (can be done under vacuum).
 11. Embed in pure epon at 37° C for 24 hours.
 12. Cure for 48 hours at 60° C.
- Blocks are now ready to section, stain, and scope.