Dewan/Loomis-Protocol: Revised 12-16-2016

Tissue preparation and cryopreservation with sucrose -- for frozen tissue sections

The purpose of cryopreserving tissues is to help prevent ice crystal formation in tissues when water freezes and expands. Ice crystals break cell membranes and produce holes within cells and loose extracellular matrix ("Swiss Cheese" artifacts). The two basic strategies for preventing crystals during the transition from water to ice are: 1) freeze as rapidly as possible (seconds); and 2) add cryoprotectants that disrupt interactions between polar water molecules. Cryoprotectants are particularly important when freezing large tissue samples such as rodent brains. Common cryoprotectants used to preserve tissue morphology include sucrose, glycerol and polyethylene glycol.

Protocol for cryopreservation with sucrose

Do all steps at 4°.

1. After removal of the tissues from the body, wash briefly in ice cold PBS plus Ca++ and Mg++ (Ref.: 21-030-CV, Mediatech Inc.).
2. Fix tissues in fresh (<1wk old) 4% "paraformaldehyde" (e.g. Cat # 15714, Electron Microscopy Sciences for 32% stock) at 4°C (see instructions for PFA prep or Dilute 8 times with 1xPBS to make 4% PFA) or 10% neutral buffered formalin (Cat # SF100-4, Fisher Scientific). The most ideal form of fixation for animal organs involves transcardiac perfusion of PFA prior to removal of the organ from the body (see "Fixation" and "Perfusion" protocols). Time of subsequent immersion fixation depends on subsequent steps, but the best morphology is obtained if they are fixed 24 hrs after perfusion or 48-72 hrs if only immersion fixed. See notes below re situations when the tissues cannot be fixed for long periods.
3. Place tissues in 15% sucrose (Cat # S5-3, Fisher Scientific) in PBS until tissue sinks (6-12 hrs) and then 30% sucrose in PBS for overnight or until tissue sinks. Best if the tissues are gently nutated, taking care to avoid contact with bubbles and the air surface interface. See notes below if not fully fixing tissues prior to cryopreservation.
4. Embed tissue in OCT or Tissue Tek or NEG 50™ (Cat # 22—110-617, Fisher Scientific), as described in the cryo-embedding protocol.

Trouble shooting and Notes:

Tissues should be well-fixed with a formaldehyde-based fixative prior to cryopreservation with sucrose because sucrose solutions above 10% are hypertonic and will cause water to flow out of cells and tissue shrinkage if tissues are not fully fixed. A short period of fixation prior to cryopreservation with sucrose can be used IF: 1) immunostaining an epitope that is sensitive to cross-linking; or 2) assaying enzyme activity that is killed with excessive cross-linking. If staining for B-galactosidase activity, fix for short periods (30 minutes – 1 hr) if using 4% PFA. (See lacZ – β-galactosidase staining protocol for an alternative fixative that allows longer times). For immunostaining, some antibodies can only tolerate 10-15 mins of cross-linking fixative whereas others can tolerate 24 hrs or more. This must be worked out for each antibody. Note, if tissues are only partially fixed, times in sucrose should be reduced.

Some tissues can be put directly into 30% sucrose, but the pressure caused by the osmolarity differential across the cell membranes has significant negative impact on tissue morphology for most tissues. For very fragile tissues and for GFP visualization, some labs use 20% instead of 30% sucrose.

Frozen sections are much more fragile than paraffin sections and they do not tolerate harsh antigen retrieval protocols. As a result, freshly made PFA is often used instead of formalin for fixing frozen tissues because the extent of cross-linking is more consistent.