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Cryoprotectant Tissue Storage Solutions: Stability at Lower Temperatures, Longer Storage Times, More Versatile Usage

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Infusion with buffer/sucrose solutions (up to 30% sucrose) has long been used to 'cryoprotect' tissue in an attempt to prevent ice crystal artifact in frozen sections. This is helpful for example in sectioning large, fixed tissue blocks that must be frozen relatively slowly in dry ice to allow sectioning on a sliding microtome. In 1977, De Olmos[1] added ethylene glycol to the mixture (30 g cane sugar/50 ml 0.1M PO_4 buffer at pH 7.2 in 20 ml ethylene glycol) for -10°C storage of free floating sections from lightly fixed primate brain. Jones and Kane in '78[2] used this solution for storage of sections at -20°C (standard household freezer temperature) for up to one month before horseradish peroxidase histochemical reaction. In their methods, they cautioned that "sucrose attracts insects" (ants, personal communication). We and others have found the above cryoprotectant solution generally useful for storage of free floating sections from fixed brain. We observed that adjacent sections stored at 4°C in buffer for 2 weeks (common practice for Nissl stained sections) lost their reactivity to antibody labelling. However in contrast, sections stored in cryoprotectant at -20°C retained their antigenicity for months, sometimes for years!

For studies using immunohistochemistry and cRNA *in situ* hybridization in sections cut from the same tissue block,[3] we wanted to eliminate all sources of contaminating RNAses. Therefore, we modified our standard cryoprotectant formula to eliminate sucrose (cane sugar) and to contain only RNAse-free molecular biology grade reagents:

20% glycerol
30% ethylene glycol
50% 0.1M phosphate buffer, pH 7.0-7.4

This formulation is more costly (especially when using molecular biology grade reagents), but well worth it for our purposes. If you are not concerned about proteases or RNAases, I suspect that with standard lab grade chemicals, the benefits (ease of preparation in large or small amounts, safety and stability, freedom from ants...) outweigh most of the increased expense.

One feature of the above formulation is that it does not freeze at -30°C or -35°C (as with sucrose formulae), but does freeze at -70°C or on dry ice. We have found this feature useful for shipping free floating sections stored in 24 well tissue culture trays, as well as uncut blocks of tissue in vials of cryoprotectant. Even though the solution with tissue freezes, the cryoprotectant prevents ice crystal artifact and loss of antigenicity or mRNA. We have not tested between -35°C and -70°C, so I don't know the exact freezing point of the solution.

Cryoprotectant-fixative Tissue Storage Solution: Deleterious effects of over-fixation for immunohistochemistry are well known, and we usually guard against it for our research material. However, we have observed that additional aldehyde fixation is actually preferable for optimal labelling of mRNAs by in situ hybridization. In addition, we discovered that sections to be used only for *in situ* hybridization benefited from storage immediately after sectioning in the above cryoprotectant solution containing 2% paraformaldehyde. In fact, controlled tests showed the signal-to-noise ratio for ^{35}S *in situ* hybridization autoradiographs[4], (and unpublished observations) was significantly improved. To obtain a final concentration of 2% paraformaldehyde, 4g granular paraformaldehyde is dissolved using heat in each 100 ml of the constituent 0.1M phosphate buffer before adding it to the cryoprotectant solution. ■

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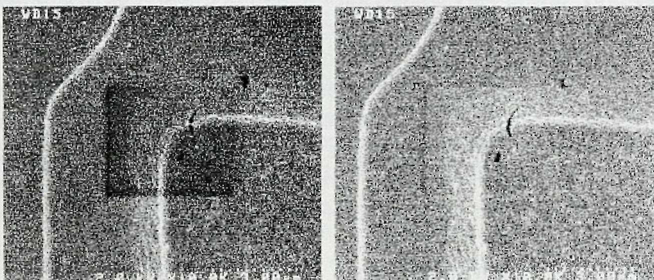
Remove Contamination from SEM Samples *in-situ!*



A contamination deposit from a short SEM scan. | Flash SEM-CLEAN removed most of the deposit in 2 minutes.

Flash SEM-CLEAN "plasma" Cleaning

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