CRYOPROTECTANT SOLUTION (SOP-19)

This solution was originally developed by J. S. de Olmos for preserving HRP reactivity in brain sections. This solution also preserves immunoreactivity within cut sections for at least 3-6 months, and in some cases actually appears to improve the quality of staining. Preliminary observations suggest that this solution also may be useful for storing blocks of tissue and for preserving general tissue morphology for EM applications (Watson et. al., 1986, Peptides, 7:155).

FOR 1000 ML OF CRYOPROTECTANT:

500 ml	100 mM phosphate buffer (pH 7.2)
300 g	sucrose (30% w/v)
10 g	polyvinylpyrolidone (PVP-40, Sigma, 1% w/v -- optional)
300 ml	*ethylene glycol (30% v/v)

Adjust final volume to 1000 ml with dH_2O.

Frozen or vibratome sections (25-100μ) should be cut into compartmentalized baskets or MULTIWELL™ Tissue Culture Plates (Falcon 3047) containing cryoprotectant solution. The solution should be cold (~4°C) and the collecting tray kept on ice while the tissue is being cut. Store tissue overnight at 4°C, then transfer to the freezer (-20°C) for long term storage.

HRP reactivity may begin to decline after 2-4 weeks of storage, especially if the fixative contained <1% glutaraldehyde. There is no appreciable decrease in immunoreactivity for at least 3 months. Longer storage is possible, with tissue stored more than 1 year still showing robust immunoreactivity. However, the possibility of some slight loss of immunoreactivity has not been systematically evaluated at these longer storage times.