Introduction

Methods based on the Tokuyasu thawed cryo-section technique are the most sensitive post-sectioning methods for immunolabelling, with the initial aldehyde fixation stage being the only chemical denaturation step.

The first stage in any new IEM study is to determine the effect(s) of the fixation method on antigenicity. This is done by immunofluorescence (IFA) using (para)formaldehyde fixed and permeablized material. Apart from assessing the effects of fixation on the antigen, this approach also provides some idea of the localization and signal that you might expect at the EM level. In this way, you can establish the range of antibody dilutions most useful for localization at the EM level. However, antibodies that produce poor signals by LM do not improve at the increased level of resolution of the EM.

Protocol

Start fixation in 4% FA in 250 mM Hepes buffer (pH 7.4) for ~30 min at RT then transfer to fresh 8% FA in 250 mM Hepes buffer (pH 7.4)

Continue fixation for 2-24 h at 4ºC.

Wash the cells/specimen in 250 mM Hepes and then in 250 mM Hepes with 20 mM glycine (to block any free aldehyde groups) - >30 min at 4ºC.

Scrape cells from the plate(s) and gently pellet and remove Hepes/glycine solution and gentle wash in Hepes. Re-suspend the cells in warm 2% gelatin in PBS and allow the gelatin to infuse the cells (~15 min without allowing the gelatin to harden) and then gentle pellet into the gelatin. Change the gelatin, re-suspend the cells and repeat.

Allow the gelatin to harden at 4ºC and then free the gelatin-embedded pellets from the tubes using a cocktail stick and cut into ~0.5 mm cubes. Gelatin-embedded cells were then infused with 2.3 M sucrose in PBS (overnight at 4ºC) and mounted on to holders and frozen in LN2.

For tissue samples it is not necessary to embed them in gelatin and, once they have been blocked with 250 mM Hepes - 20 mM glycine, the samples are infused with 2.3 M sucrose (overnight at 4ºC) and small blocks mounted and frozen.

Once frozen, the specimens are stored (indefinitely) under LN2.