Preparation of pellets for cryosectioning

- 4% paraformaldehyde (PFA) – most antibodies work with this
- 4% PFA, 0.1% glut. – ultra-structure much better with this

Use one dish of 10cm diameter for each condition. Just remove media, rinse once with PBS and then add the fixative.

Fix (4% PFA, 0.1% glut. or 4% PFA) in 0.1M phosphate buffer pH 7.35, 1h RT.

Note: use EM grade commercial paraformaldehyde and glutaraldehyde, best are the sealed ampoules you can buy.

After fixing at RT, wash with PBS then with PBS/50mM glycine, then scrape cells. Spin down (10K microfuge; not faster, since this ruins morphology)

If you want you can stop at this point and send the cells, otherwise preferably:

1. remove supernatant.
2. add warm 10% gelatin (NOT fish skin gelatin – it should be food grade gelatin) in PBS (should be completely dissolved and clear) and fill tube to top (NB. Pre-spin 10% gelatin before use to get rid of aggregates).
3. re-suspend pellet carefully – eg. with blue Gilson tip with end cut off so cells aren't damaged – the cells don't have to be dispersed – just have to have gelatin all around them.
4. spin max speed microcentrifuge.
5. remove some of the gelatin leaving it just over pellet.
6. cool on ice until gelatin is solid.
7. add PBS to tube and fill up to top.

There should be a nice, visible, concentrated pellet in the bottom of the tube. If it is slightly up the side of the tube this is no problem, but it's not good if it's smeared all the way along the tube.