

Harvesting of solid tissue cultures

1. To each culture add colcemid (working solution 0.1mg/ml). Use 0.1ml per 1ml of culture medium.
2. Place the flasks in the incubator for 1.5 - 2 hours
3. To the flasks add 0.5ml – 1ml Trypsin-EDTA (1x) and incubate for 3-4 mins.
4. Examine each flask using the inverted microscope. The cells should be "rounding up" off the bottom of the flask. Tap the flask firmly several times. Examine the flasks; the cells should be in suspension
5. Transfer the cell suspension from each flask to a labelled centrifuge tube using a sterile plastic pipette.
6. Centrifuge the tubes at 1200 r.p.m. for 7 minutes.
7. In the class II cabinet, pour off the supernatant from each tube into a beaker containing 2% Virkon solution. Resuspend the pellet by flicking the tube.
8. Add 5ml **KCl hypotonic solution (0.055M)** to each tube, gently invert twice to mix. Centrifuge at 1200r.p.m. for 7 minutes.
9. In the class II cabinet, pour off the hypotonic from each tube into a beaker of 2% Virkon. Remove excess moisture using by tapping the tube rim on a paper towel, and then thoroughly resuspend the pellet by flicking the tube.
10. Prepare the fixative in a fume cabinet, 3:1 Methanol:Acetic acid.
11. In the fume hood, add the fixative **dropwise to the side** of the tube using a plastic pipette. Add about 3 drops and allow this to run down the side of the tube as you agitate it. This step is important because if not performed correctly the cells may clump together. After approx. 0.5ml has been added, top up to 3ml. Centrifuge at 1200 r.p.m for 7 mins.
12. Fixed cell suspension can be stored at -20°C or sent to the Cytogenetics Laboratory for slide making.