

# Ethanol fixation for flow cytometry

Fixation for flow cytometry with ethanol. This method is very simple, quick and allows samples to be stored for weeks at  $-20^{\circ}\text{C}$ .

- From a 35mm-10cm plate harvest cells with trypsin into a 15ml tube.

*Keep media as this helps to inactivate trypsin and many mitotic and dead cells will be floating in the media*

- Pre-chill 100%  $-20^{\circ}\text{C}$  high grade Ethanol
- Centrifuge at low speed 1500rpm 10 minutes to pellet
- Remove supernatant media and resuspend in 1ml PBS
- Transfer to a 1.5ml eppendorf
- Centrifuge at 5000rpm for 2-5min to pellet
- IMPORTANT: Remove supernatant PBS and gently resuspend pellet in 300ul of PBS
- Add 700ul of 100%  $-20^{\circ}\text{C}$  high grade Ethanol (EtOH) inverting tube a few times to ensure proper mixing and a good fixing of cells.
- Store at  $-20^{\circ}\text{C}$  until required for flow cytometry/FACS