Freezing Cells

Procedure

1. Thaw FBS, DMSO, Prepare freezing medium, 70% DMEM, 20% FBS, 10% DMSO (7ml, 2ml, 1ml for 10 ml medium), keep in 4^{o}C. (Note: DMSO is easy to decompose. It should be kept in fridge and avoid light by covering with alumn foils. To desolve DMSO, it should be kept in room temperature, not in warm water bath. And the freezing medium is only good for a 1-2 days, so it should be prepared right before experiment.)
2. Rinse confluent cells with PBS for 3 times
3. Incubate cells with 2 ml 0.5x trypsin (10mm dish) (1x for smooth muscle cells and 0.5x for endothelial cells), keep in 37^{o}C for 1.5 min, not to over 2 min.
4. Gently pat the dish, check under microscopy to make sure all the cells are in suspension.
5. Quickly add DMEM (must contain Calcium), collect cell solution into a tube and centrifuge 1500rpm for 3 min. (keep the balance of centrifuge).
6. During the centrifuging period, Label the freeze epitaphs with cell name, passage, date, initials of your name.
7. Take out the centrifuged tube containing cells, you should be able to see a whitish pellet at the bottom of the tube. Tilt the tube and aspirate the supernatant with vacuum tip, re-suspend the cell pellet with Freezing medium by pipetting up and down 20 times to break cell-cell aggregation. Apply cell solution to labeled epitaphs.
8. Freeze in -70^{o}C in the Cryobox originally at RT for overnight.
9. Transfer the tubes to liquid nitrogen tank the next day, register in the log book.

Notes

- 1 large dish to 6 epitaphs (1.5ml each)

- 10% DMSO for MEF, HeLa, MDA-MB-231; 5% for SK-BR-3 cells.