CRYOPRESERVATION OF SF9/H5 CELLS

Materials and reagents:

1. Dimethyl sulfoxide DMSO (Fluka; cat. #41650)
2. Fresh serum-free insect media (Expression Systems; ESF 921, cat. #96-001-01)
3. Conditioned serum-free media (pre-frozen media or media from the cells you are freezing)
4. Trypan Blue 0.4% solution (Sigma; T8154)
5. 0.22uM sterile filter
6. 50ml syringe
7. 4 x 50ml tubes
8. Labeled cryo tubes
9. Ice bucket
10. Cryo-tube rack to fit into the ice bucket

Tips:

This procedure is for cells growing in suspension, in serum free media. The cells should be in an excellent shape: doubling every 24h, and less than 3% dead when counted with trypan blue. Cell should be in exponential growth stage, and not used if grown over 4 million/ml, or less than 2 million cells/ml.

Instructions:

1. On the prior day, (preferably in the morning) dilute suspension cells to 1-1.5 million cells/ml in 100ml
2. Count cells and viability before starting. Only viable culture >97% should be used. (If cells doubled, and now reach 2-4 million cells/ml, they are suitable for freezing):
3. Transfer cells to 2 x 50ml tubes and spin down in centrifuge at 100 g, for 5min at 22°C.
4. Remove media from cells, (keep it if you wish to use it for preparation of freezing media)
5. Prepare freezing media:
   a. 7.5% DMSO
   b. 46.25% fresh serum-free media
   c. 46.25% conditioned media
   d. Mix all components in a 50ml tube, transfer into 50ml syringe and filter though 0.22uM sterile filter into a new sterile 50ml tube.

e. Place the freezing media on ice, and chill it to 4°C

5. Gently re-suspend cells in chilled freezing media to a final concentration of 2-4 x107 cells/ml by pipettation. (When freezing 100ml suspension growth, we usually re-suspend the cells in 10ml freezing media.)
6. Aliquote cells to 1.5ml portions in a pre-labeled 2ml sterile cryotube.
7. Keep cryotube vials on ice for 30 min.
8. Transfer vials to –70oC for an over-night storage.
9. Next day, transfer tubes into liquid nitrogen storage tank.