Insect Cell Cryopreservation

Freezing S9, Sf21, S2 and Tni cells:
Reagents:

Freezing Medium Part I: 80%ESF-921, 20% DMSO
Freezing Medium Part II: 0.3M Trehalose in ESF-921 Medium
Combine Part I and Part II at 1:1, sterile filter and keep at 4° C for up to 1 month.

Alternatively, cells can be frozen in 90% ESF 921, 10% DMSO. ESF AF can also be used in place of ESF 921.

Cell Freezing:

1. Insect cells to be frozen should be in log phase prior to freezing.
2. Count cells and transfer desired amount to 50 ml conical.
3. Spin cells at 1000 rpm for 5 minutes in sterile centrifuge tube.
4. Gently resuspend the cell pellet with freezing medium. Final concentration should be between 25 – 50 x 10^6 cells per ml.
5. Place 1 ml cell suspension in pre labeled cryo vials
6. Keep cells on ice as much as possible
7. Move vials to a Nalgene Cryo 1ºC Cooling container and place at -80ºC for 24 hours. (The use of a controlled freezing vessel is optional but strongly suggested).
8. Transfer vials to Liquid Nitrogen storage container

Cell Recovery:

1. Place 50 ml ESF 921 into 125 ml shake flask.
2. Obtain cells from the liquid Nitrogen storage
3. Rapidly thaw tube of frozen cells in a water bath. Shake tube in water bath until the sample begins to thaw. There should still be some frozen material in the tube. Wash vial with ethanol before opening. Use a 1 or 2 ml pipet to transfer contents of tube to flask.
4. Place flask in incubator at 27 C. Shake speed should be approximately 140 rpm (speeds will vary depending on incubator). Cap should be loosened to allow for gas exchange.
5. Sample flask 24 hours later for count and viability. The count should be between 1 and 2 x 10^6 cells per ml and the viability should be greater than 90%. Transfer the cells to the flask from step 1 and place in incubator.
6. Cells should be ready to split after 72-96 hours. Passage cells every 3-4 days.