Freezing and Thawing T or B cell hybridomas

Freezing Mixture (FM):
55% MEM
30% Fetal Bovine Serum
15% DMSO (sterilize by autoclaving)

Spin down ~ 5 x 10^6 cells. Resuspend in 0.5 ml complete medium. Transfer into a
cryoivial. Add 0.5 ml FM. Mix well. Transfer the cryovial into a Styrofoam rack
(both top & bottom) and place in −80°C freezer. After 24 hours the vials can be
transferred into the liquid nitrogen freezer.
** If your B cells are very adherent to the flask they should be scraped from the
flask with a disposable scraper.

Thawing cells:
Place frozen vial in 37°C briefly. Transfer the contents of the vial into a 15 ml
conical tube containing 3 mls balanced salt solution.
Underlay ~ 2 mls Fetal Bovine Serum under the cell mixture.
Spin 5 minutes, 1500 rpm (500 g), Beckman table top.
Suction off all of the medium and fetal bovine serum. (The DMSO stays on top of
the FBS).
Resuspend the pellet of cells in 30 mls Complete Tumor Medium in a T-75 flask.