

## Freezing and Thawing T or B cell hybridomas

### **Freezing Mixture (FM):**

**55% MEM**

**30% Fetal Bovine Serum**

**15% DMSO (sterilize by autoclaving)**

**Spin down ~  $5 \times 10^6$  cells. Resuspend in 0.5 ml complete medium. Transfer into a cryovial. Add 0.5 ml FM. Mix well. Transfer the cryovial into a Styrofoam rack (both top & bottom) and place in  $-80^\circ$  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  $37^\circ$ 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.**