

## Freezing and Cryopreservation of Human Lymphoblastoid Cell Lines

Note: This protocol requires use of sterile technique in a TC hood.

- The day before, add 10ml of complete media to each of the flasks containing the cells to be frozen so the final volume is 30 mls.
  - The day before, prepare for the freeze by setting up your tubes, generating your labels and filing out your freeze sheet.
- 1) Pre-warm your complete RPMI-1640 to room temperature.
  - 2) Prepare the freezing medium by adding DMSO to a final of 7% and gently mix. Note: prepare at least 6 ml of media for each cell line being frozen.
  - 3) Transfer the contents of your T75 flask into 50 ml pre-labeled conical polypropylene centrifuge tubes.
  - 4) Pellet the cells in a centrifuge at 3000 RPM for 10 minutes (Program #4).
  - 5) Carefully decant the supernatant into a waste container containing bleach.
  - 6) If the cells from a single culture are in two separate 50 ml tubes, combine the pellets with complete RPMI-1640 and re-pellet and decant as in steps 4 and 5.
  - 7) Resuspend the cells into 6 ml of freezing medium.
  - 8) Aliquot 1 ml of the cells into six labeled 1.8 ml cryovials (Nunc: Cat. No. 377267).
  - 9) Transfer the cryovials to a Cryo Freezing Container (Nalgene Cat. No. 5100-0001) and place at  $-80^{\circ}\text{C}$  overnight. Note: when filled with isopropanol, the freezing container will provide the desired freezing rate of  $1^{\circ}\text{C}/\text{minute}$ .
  - 10) Transfer the vials from the freezing container to long-term storage in a liquid Nitrogen freezer. Four tubes go into the main freezer, and 2 go into the reserve.
  - 11) Record the necessary information into catalog or database.

### **RPMI-1640 (complete)**

500 ml of RPMI-1640

100 ml heat-inactivated FBS (20%) – Gibco/Invitrogen: Cat. No. 16140-071

6 ml antibiotic-antimycotic (1x) – Gibco/Invitrogen: Cat. No. 15240-062

1 ml Tylosin solution – Sigma-Aldrich: Cat. No. T3397

### **Freezing Media (prepare fresh)**

Complete RPMI-1640 (20% FBS) with 7% DMSO

Prepare enough for at least 6 ml per cell line being frozen.

(DMSO: Fisher: Cat. No. BP231-1)

### **Other Items Needed**

Isopropanol