Freezing and Cryopreservation of Human Lymphoblastoid Cell Lines

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

The day prior to freezing:

- Add 10 mL of complete media to each of the flasks containing the cells to be frozen so the final volume is 30 mL.
- Arrange the flasks by ascending number and label them 1, 2, 3,…
- Prepare for the freeze by setting up your tubes, generating your labels and filing out your freeze list.
- Create your aliquots in LabMatrix (see GMB SOP #), print them out and prepare a freeze Cell Line Freeze Log.
- Place the appropriate colored inserts into the tubes and label them, arranging 3 sets of 6 tubes per numbered rack (the racks are numbered to correspond to the Cryo-Freezing Containers.

All pipettes are disposed of by placing in a biohazard was container. All liquid is discarded by placing into a waste container containing bleach, which will serve to decontaminate the media.

**Once you are done, decontaminate the collected liquid waste by adding 10% Clorox solution and allow to sit for 15 minutes. After 15 minutes, the decontaminated waste may be flushed down the sink with water.*

1) Pre-warm complete RPMI-1640 to room temperature.
2) Prepare the freezing medium by adding DMSO to a final of 7% and gently mix. Note: prepare 6 mL of media for each cell line being frozen.
3) Transfer the contents of your T75 flask into 50 mL pre-labeled (1, 2, 3,…) conical polypropylene centrifuge tubes. Be sure to transfer the sample label as well.
4) Pellet the cells in a centrifuge at 3000 RPM for 10 minutes (Program #4).
5) Carefully decant the supernatant into a waste container.*
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 in 6 mL of freshly prepared freezing medium.
8) Aliquot 1 mL of the cell suspension into six labeled 1.8 ml cryovials *(Nunc: Cat. No. 377267).*
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10) Wearing a face shield, transfer the cryovials to the correct Cryo-Freezing Container *(Nalgene Cat. No. 5100-0001)* and place at –80° C overnight. Note: when filled with isopropanol, the freezing container will provide the desired freezing rate of 1° C/minute.

- You will have a maximum of 3 sets of 6 vials in each tube rack.
- Fill the set in the front of the rack first.
- This set will be the first to go into the Cryo-Freeze Container and you place it in the middle of concentric circle. The other 2 sets will be placed around the first set.
- This allows the person archiving the tubes in the liquid nitrogen to know which set comes first.

11) Wearing a face shield, 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 into the reserve.
12) Record the necessary information on to the Cell Line Freeze Log then into the database.

RPMI-1640 (complete)
500 mL of RPMI-1640
100 mL heat-inactivated FBS (20%)
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