



Title: *Freezing and Thawing Cell Lines*

No: RTLP-GLP-CP6

Location:
Old CCRC Tripp Lab

Approval Date:
13 July 2005

Supersedes Date:

Materials:

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- | | | | |
|------------------------|---------------------------|-----------------|------------------|
| •Lab coat | •DMEM | •Tissue culture | •Pipettes |
| •Gloves | •37°C, 5% CO ₂ | flasks | •Pipetteman |
| •0.5% trypsin-EDTA/4Na | Incubator | •Centrifuge | •Pipette Aid |
| • Fetal bovine serum | •DMSO | | •Pipetteman tips |

Procedure:

NOTE: Rule of thumb is “**Slow freeze, Quick thaw**”. This means cells to be put in LN₂ should *first be slowly frozen* at -70°C. When thawing cells, place the vial in 37°C H₂O bath to quickly thaw.

Procedure:

Freezing Cells

1. Harvest cells to be frozen and pellet by centrifugation at 2000 rpm for 10 minutes at room temperature.
2. Resuspend pellet in freezing media (92% Fetal Bovine Serum + 8% DMSO).
3. Place cells into -70°C freezer for slow overnight freeze. The next day place into LN₂ storage.

Thawing Cells

1. Take vial of cells out of LN₂ storage.
2. Quickly thaw cells by placing into 37°C H₂O bath - monitor their thaw.
3. Place cells into 15mL centrifuge tube and bring up to 10 mL with 0% DMEM

4. Wash cells by centrifugation at 2000 rpm for 10 minutes at room temperature (this step is required to remove DMSO).
5. Resuspend cells in desired growth media and check viability at the inverted scope. If cells look healthy, they can be placed into T-75 flask from this thaw. If cells look unhealthy, it may be best to seed in a T-25 flask (or in some desperate cases, a 24-well plate). In some cases, it may be necessary to place over a lymphocyte gradient to pellet dead cells from live ones. If in doubt, ask Rene or Chris for help.