

**SOP: AB105**

**Freezing Down Cell Lines**

**Materials and Reagents:**

1. RPMI 1640 medium with L-glutamine, without sodium bicarbonate (Invitrogen 11875-093)
2. Fetal Calf Serum (FCS)
3. DMSO tissue culture grade
4. CO<sub>2</sub>(5%) Humidified Tissue Culture Incubator 37°C
5. 50 ml falcon centrifuge tubes
6. 15 ml falcon centrifuge tubes
7. Hemocytometer
8. Serological pipettes (1 and 5 ml)
9. Tissue culture/biosafety hood
10. Cryovials
11. Cryovial storage container
12. -80°C Freezer
13. Liquid Nitrogen storage
14. Ice bucket
15. Table top centrifuge
16. 0.2 µm filter (Nalgene)
17. Inverted Microscope

**Protocol:**

1. \_\_\_\_ Look at cells using an inverted microscope (note 1).
2. \_\_\_\_ Count cells with hemocytometer (note 2).
3. \_\_\_\_ If cells are in normal density range, pellet them in a sterile centrifuge tube by spinning between 1200 – 1500 rpm for 10 minutes.
4. \_\_\_\_ While cells are spinning, label cryovials with appropriate information and place in ice bucket (note 3).
5. \_\_\_\_ In a tissue culture hood, remove supernatant carefully with a pipet and discard.
6. \_\_\_\_ Tap bottom of tube to break up cells and resuspend cells in appropriate amount of cold Freezing Media to obtain necessary density (note 4 and 5).
7. \_\_\_\_ Immediately transfer cell suspension to appropriately labeled cryovial and put on ice until it can be frozen (note 6).
8. \_\_\_\_ As quickly as possible, transfer cells to -80°C freezer. Let sit overnight (note 7).
9. \_\_\_\_ After 24 hours, the cell line may be transferred to liquid nitrogen storage. Make note of location in LN2 log book and in project notebook (note 8).
10. \_\_\_\_ After at least a day, thaw one vial from each lot of frozen cells to check viability (note 9).

**Notes:**

1. Cells should appear >90% viable. Any lower and the cells may not be able to recover from the freezing process.
2. For suspension cultures, cell density should be between  $3 \times 10^5$  and  $7 \times 10^5$  cells/ml.
3. Label:

<i>Cell Line</i>	<i>Date</i>
<i>Specificity</i>	<i>Media (if other than complete RPMI)</i>
<i>Cell count</i>	<i>Initials</i>

4. Freezing Media: for 200 ml

120 ml RPMI 1640 medium

60 ml of fetal calf serum (FCS) (final concentration is 30%)

20 ml dimethylsulfoxide (DMSO) (final concentration is 10%)

Filter sterilize the medium, then aliquot to 15 ml conical tubes. Store frozen at  $-20^{\circ}\text{C}$ . The media should be defrosted and placed in the ice bucket covered with ice for at least 10 minutes to ensure it is cold before use.

Do not add DMSO directly to FCS as a precipitate may occur.

5. Cells should be resuspended in 1 ml of media per cryovial, and should have a density between  $3 \times 10^6$  and  $7 \times 10^6$  cells/ml. As an example, if in 10ml, the cell count is  $4 \times 10^5$  cells/ml, then the cell count is actually  $4 \times 10^6$  cells total in the flask. So after pelleting, the cells should be resuspended in 1 ml of Freezing Media to maintain this density.

6. The cells should stay on ice for as little time as possible.

7. The cells should never be stored at  $-20^{\circ}\text{C}$  for any period of time. Do not transfer cells directly to LN2 tank, as this will be too great of a temperature change and the viability will be low, or the difference in temperature will cause the lids to pop off and the line will have to be discarded.

8. Cell viability decreases from 1-10% per month if the cells are kept at  $-80^{\circ}\text{C}$ .

9. See SOP:AB100 for Cell Line Maintenance.