# Cryopreservation of Cell Lines

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This protocol describes how to freeze down a cell line for cryopreservation. Give proper attention to good sterile technique at all times. It is written from a basis of approximately 10 million adherent cells in one T175 flask. Scale accordingly.

1. Prepare freezing medium and container
    a. Prepare 12 mL of freezing medium for every 10 million cells to be frozen, which is typically ~one T175 flask with at most 75% confluence (by area in phase contrast). Scale from a 14 mL sterile conical tube as necessary.
    b. Mix 600 uL (5%) sterile DMSO (Sigma-Aldrich D8418), 6 mL (50%) full growth medium, and 5.4 mL (45%) fetal bovine serum (BioTC FBS-02) (v/v/v). Use full growth medium from the cell line of interest.
    c. Find “Mr. Frosty” (Thermo-Fisher 5100-0001) and warm to room temperature if needed. Fill with isopropanol to the fill line.
        i. If someone has left “Mr. Frosty” in the -80$^{o}$C freezer with cells for a long period of time, you may berate them. But please don’t just throw their cells out, put them in the liquid nitrogen if you can’t find the responsible person.
2. Harvest and count cells
    a. Cells should be subconfluent (< 75% by area in phase contrast) and thus in exponential growth phase.
    b. Lift cells according to the cell line-specific subculture details.
    c. Pellet cells (typically 100g, 5 min), aspirate supernatant, and add 3 mL of freezing medium. Resuspend with a 1000 uL micropipette tip with repeated pipetting to minimize cell clumping.
    d. Count cells using a hemacytometer (or alternative method of choice).
    e. Scale cell density to 1 million cells / mL with freezing medium.

3. Aliquot and freeze cells
    a. Prepare 10 cryovials (Nunc 377267) by loosening their screw tops under the sterile hood.
    b. Transfer 1 mL of the cell suspension to each cryovial and close tightly.
        i. We use a 1000 uL micropipette to improve consistency and reduce cell clumping.
    c. Record on the cryovial cell line name, passage number, and date.
        i. This step allows for a reasonable time delay (15 min or so) recommended before transfer to -80$^{o}$C.
    d. Place cryovials into “Mr. Frosty” and to the -80$^{o}$C freezer overnight.
4. Transfer cells to liquid nitrogen
    a. The next day, take cells from -80$^{o}$C storage and place on dry ice.
    b. Place cryovials into available box space in the liquid nitrogen storage. Record the cane number, box number, and positions within the box.
    c. Update LabGuru records and return Mr. Frosty to the designated location.