

Technical Notes—How to Freeze Cell Products

The below information is for customers who have extra fresh cell products or have fresh cells that are not needed immediately for processing. Please note, freezing cells risks poorer viability and poorer cell recovery after thaw.

How to Freeze Cell Products

(with the exception of bone marrow mononuclear cells)

Reagent and Materials Required

- Fetal Bovine Serum (FBS)
- Dimethyl Sulfoxide (DMSO)
- Isocove's Modified Dulbecco's Medium (IMDM)
- Ice

Equipment Required

- Refrigerated centrifuge
- Biosafety hood (Class II)
- Compound microscope
- 2mL Cryogenic vials
- Freezing rack
- 1 to 10 mL pipets
- Conical centrifuge tubes
- Isopropanol graded freezing containers
- -20°C freezer
- -80°C freezer

Procedure

1. Label cryogenic vials with the appropriate vial and donor information.
2. Place ice at the bottom of a freezing rack.
3. Place the vials in the freezing rack.
4. In a -20°C freezer, store the freezing rack until ready for use.
5. Prepare the Cryo solution. Prepare enough Cryo solution for the number of vials needed to freeze.
 - a. For Cryo solution combine: 80% FBS and 20% DMSO (Note: The final percentage of the cryopreservation media is 50% IMDM, 40% FBS, 10% DMSO).
 - b. For cell concentrations that are > 1 million, the recommended final volume is 1.8mL. The ratio of cell suspension to Cryo should be 1:1. This means that there should be 0.9mL of Cryo for every vial.
 - c. For cell concentrations that are less than 1 million, the recommended final volume is 1.2 mL. The ratio of cell suspension to Cryo should be 1:1. This means there should be 0.6 mL of Cryo for every vial.
 - d. Combine DMSO and FBS. Mix well and leave the solution on ice for at least 10 minutes.
6. The cells should be spun down into a pellet in the centrifuge.
7. Resuspend the cell pellet in IMDM.
 - a. Resuspend large concentrations of cells ($> 1 \times 10^6$ /vial) at 0.9mL per vial.
 - b. For smaller cell concentrations of cells ($< 1 \times 10^6$ /vial) at 0.6mL per vial.
8. Mix the suspension well. Chill the cells on ice for approximately 5 to 10 minutes.
9. In a small bowl, add ice and enough water to make a homogenous ice-water mixture.
10. Place the cell suspension tube in the ice-water mix. Add the same amount of Cryo solution as the cell suspension (1:1) into the cell suspension at about 3 to 5 seconds per drop. Shake the tube constantly to ensure even mixing.
11. Take out the freezing rack from the freezer and place it in the hood. Mix the cells well with a pipet and add the mixture to the cryogenic vials.
12. Add the appropriate amount.
 - a. 1.8 mL for cell concentrations greater than 1×10^6 /vial.
 - b. 1.2 mL for cell concentrations less than 1×10^6 /vial.
13. Place vials in an alcohol-graded freezing container(s) and place in a -80°C freezer. (The isopropanol allows the vial temperature to decrease at 1°C/min.)
14. Freeze over night. For long-term storage (> 1 month), we recommend moving the vials to the vapor phase of liquid-nitrogen storage.