

Technical Notes—How to Freeze Cell Products—Bone Marrow-MNC

How to Freeze Bone Marrow MNC

Reagents Required

- Washing Buffer: PBS (Ca^{2+} and Mg^{2+} free) with 2mM EDTA and 10% FBS
- Cryostor™ CS5
- Fetal Bovine Serum (FBS)
- Ice

Equipment Required

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

Procedure

1. Determine the number of vials needed and the concentration of freeze.
 - a. Concentration of cells $\leq 1 \times 10^6$ freeze at 1.2ml
 - b. Concentration of cells $> 1 \times 10^6$ freeze at 1.8ml
2. Centrifuge the cells in a 50ml conical tube at 200xg at 4°C for 10 minutes without break. Remove the supernatant.
3. Use Cryostor™ CS5. Resuspend a small amount of Cryostor CS5 with a P1000 pipettman. Bring the cell suspension to the required amount for freezing.
4. Aliquot the appropriate amounts of cell suspension in the cryogenic vials for freeze.
5. Place vials in isopropanol graded freezing containers and place in a -80 °C freezer. For long-term storage (> 1 month), we recommend moving vials to the vapor phase of liquid-nitrogen storage.