Jan 14, 2026

Cryopreservation of Mammalian Cells in a Mr. Frosty Cell Freezing Container

DOI
https://dx.doi.org/10.17504/protocols.io.81wgbox63lpk/v1

Mannan Nouri[1,2]

[1]Harvard Medical School; [2]Beth Israel Deaconess Medical Center

Mannan Nouri
Harvard Medical School, Beth Israel Deaconess Medical Center

DOI: https://dx.doi.org/10.17504/protocols.io.81wgbox63lpk/v1

Protocol Citation: Mannan Nouri 2026. Cryopreservation of Mammalian Cells in a Mr. Frosty Cell Freezing Container. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbox63lpk/v1

Manuscript citation:
M. Nouri, 2024

Protocol status: Working

Created: January 14, 2026

Last Modified: January 14, 2026

Protocol Integer ID: 238581

Keywords: cell cryopreservation, cell freezing protocol, cell thawing protocol, freezing medium, cryopreservation medium, Mr. Frosty freezing container, controlled-rate freezing, long-term cell banking, cryopreservation of mammalian cell, freezing cell, dmso as the cryoprotectant, cryopreservation, rapid thaw procedure, recovery of cell, cryoprotectant, minimal freezing, cells in fresh culture medium, freezing, freezing media, free freezing media, dmso by centrifugation, plating cell, dmso, preparing serum, mammalian cell, cell

Abstract

This protocol describes how to cryopreserve adherent cell lines by preparing serum-containing or serum-free freezing media (with DMSO as the cryoprotectant), freezing cells at a controlled rate using a Mr. Frosty container, and storing long-term in liquid nitrogen or a −150 °C freezer. It also outlines a rapid thaw procedure that dilutes and removes DMSO by centrifugation before resuspending and plating cells in fresh culture medium.
Expected results are high post-thaw viability and successful reattachment/recovery of cells when DMSO is pre-mixed into freezing media and cells are handled quickly during freezing and thawing. Proper implementation should yield reproducible recovery of healthy, proliferating cultures (including drug-maintained conditions such as VCaP16 frozen with Enzalutamide), with minimal freezing-related stress or loss of cell number.

Guidelines

Prepare freezing medium before you start:

Freezing media for serum-containing medium, as follows:

- Basal medium containing 10% DMSO (dimethylsulfoxide) and FBS up to 90%.

eg: 10%-50% FBS + 16µM Enzalutmaide + 10% DMSO for VCaP16 cells.

Freezing media for serum-free medium, some of the common media constituents may be:

- 50% cell-conditioned serum-free medium with 50% fresh serum-free medium containing 7.5% DMSO
- fresh serum-free medium containing 7.5% DMSO and 10% cell culture–grade BSA.

Materials

Freezing media for serum-containing medium, as follows:

- Basal medium containing 10% DMSO (dimethylsulfoxide) and FBS up to 90%.

eg: 10%-50% FBS + 16µM Enzalutmaide + 10% DMSO for VCaP16 cells.

Freezing media for serum-free medium, some of the common media constituents may be:

- 50% cell-conditioned serum-free medium with 50% fresh serum-free medium containing 7.5% DMSO
- fresh serum-free medium containing 7.5% DMSO and 10% cell culture-grade BSA.

Materials and Equipment:

1. dimethyl sulfoxide (DMSO)
2. fetal bovine serum (FBS)
3. bovine serum albumin (BSA)
4. cell-conditioned medium
5. trypsin
6. defined trypsin inhibitor
7. centrifuge
8. cryogenic vials
9. Mr. Frosty freezing container,
10. liquid nitrogen storage
11. -150°C freezer storage
12. water bath

Safety warnings

Add DMSO to freezing media and equilibrate with pipetting BEFORE protocol. Never add DMSO directly to cell pellets or suspensions.

Work quickly once cells are in freezing medium. Get cells into tubes, tubes into Mr. Frosty and Mr. Frosty into the freezer as quickly as possible.

Prepare Freezing Medium

1 Freezing media for serum-containing medium, as follows:

- Basal medium containing 10% DMSO (dimethylsulfoxide) and FBS up to 90%.

eg: 10%-50% FBS + 16µM Enzalutmaide + 10% DMSO for VCaP16 cells.

Freezing media for serum-free medium, some of the common media constituents may be:

- 50% cell-conditioned serum-free medium with 50% fresh serum-free medium containing 7.5% DMSO
- fresh serum-free medium containing 7.5% DMSO and 10% cell culture-grade BSA.

Cell Freezing Protocol

2 Trypsinize cells for 5-10 minutes, and deactivate trypsin with serum containing medium or Defined Trypsin Inhibitor.

3 Centrifuge the cells at 200-400 G (1000-1500 RPM) for 5 min to pellet cells.

4 Using a suction pipette, remove the supernatant *without disturbing the cells_*.

5 Re-suspend cells in complete media to a pre-determined concentration for each cell line, and aliquot into cryogenic storage vials containing freezing medium (see above). Ensure final concentration of DMSO remains 10%.

6 Transfer vials to a Mr. Frosty cell freezing container, and place at -70°C to -90°C for at least 24 hours.

7 Transfer to liquid nitrogen or -150°C freezer for long term storage (>1 year).

Notes

8 Add DMSO to freezing media and equilibrate with pipetting BEFORE protocol. Never add DMSO directly to cell pellets or suspensions.

9 Work quickly once cells are in freezing medium. Get cells into tubes, tubes into Mr. Frosty and Mr. Frosty into the freezer as quickly as possible.

Cell Thawing Protocol

10 Place 13mL of culture media in a 15mL Falcon Tube.

11 Remove cryovial containing cells from liquid nitrogen or -150°C freezer.

12 Place cryovial in water bath until cell suspension is half thawed.

13 Ethanol the cryovial thoroughly and place in Biological Safety Cabinet.

14 Gently remove cell suspension from cryovial and pipette into 15mL tube containing media.

15 Spin down tube at 200-400 G (1000-1500 RPM) for three minutes.

16 Aspirate media/DMSO from tube, and gently resuspended cell pellet in suggested culture media.

17 Seed cells into appropriately sized culture plate/flask based on cell line and cell number or pellet size.