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Cryopreservation of Cells

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We use this protocol and it's working

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Abstract

This protocol outlines a systematic approach for the preservation of animal cells (especially Vero E6), aimed at maintaining cellular integrity, viability, and functionality during storage and subsequent thawing. The primary goal is to optimize long-term storage methods to support cell-based research and clinical applications

Guidelines

Store the frozen cells below –70°C; frozen cells begin to deteriorate above –50°C.

Materials

- Culture vessels containing cultured cells in log-phase of growth
- Complete growth medium, pre-warmed to 37°C
- Balanced salt solution such as Phosphate Buffered Saline (PBS), containing no calcium, magnesium, or phenol red
- Dissociation reagent such as trypsin
- Cryoprotective agent such as DMSO
- Disposable, sterile 15-mL or 50-mL conical tubes
- Equipment to determine viable and total cell counts such as hemacytometer or Coulter Counter
- Sterile cryogenic storage vials (cryovials)
- Controlled rate freezing apparatus or isopropanol chamber
- Liquid nitrogen storage container
- **Freezing medium**- 20% FBS + 10% DMSO in cell culture media

Safety warnings

DMSO at concentrations higher than 10% (v/v) is reported to induce membrane pore formation and apoptosis, thus Time is thus a critical factor when you freeze cells to minimize their exposure to DMSO.

Before start

All solutions and equipment that come in contact with the cells must be sterile. Always use proper sterile technique and work in a laminar flow hood.

1 Prepare freezing medium and store at 2° to 8°C until use

2 Remove the media from the Flask

3 Treat the cells with Trypsin/Versene solution to detach the cells from the substratum.

4 Determine the total number of cells and percent viability using a hemacytometer or cell counter

5 Resuspend the cell pellet in cold freezing medium at the recommended viable cell density for the specific cell type.

6 Dispense aliquots of the cell suspension into cryogenic storage vials.

7 Freeze the cells in a controlled rate freezing apparatus, decreasing the temperature approximately 1°C per minute. Alternatively, place the cryovials containing the cells in an isopropanol chamber and store them at –80°C overnight.

8 Transfer frozen cells to liquid nitrogen and store them