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INSTRUCTIONS FOR FREEZING CELLS

Cell viability is affected by the efficiency with which you perform certain processes. Cells are negatively affected by temperature fluctuations — severe damage to membrane and cytoskeletal components will result. Review the freezing process before initiation and move quickly and with purpose when working with these cells.

Materials Required for Freezing Cells:

- Flask with cell culture at 80-90% confluence is optimum. For best results, obtain a cell count before or during this process in order to freeze at the desired cell density.
- 5ml per T-75 culture flask of each of Passage Reagent Group™ (PRG) 1, 2 & 3
- Cell Freezing Medium
- Sterile, labeled cryogenic vials (quantity needed depends on cell density: see Step 11)
- Sterile lab gloves
- Cell culture incubator (37°C / 5% CO_2 / 95% relative humidity)
- 37°C water bath
- Ice-cold water bath (insulated bucket ¾ full of ice, plus cold water up to level of ice)
- Swing bucket centrifuge capable of maintaining internal temperature of 4°C
- 70-95% ethanol in spray bottle for disinfection of work surfaces and materials
- 15mL conical tube
- 5, 10 & 25mL serological pipettes
- Liquid waste container with sufficient capacity for the volume of waste produced

ATTAINING CONSISTENT RESULTS WITH CELL SYSTEMS

Cell Systems recommends the use of our complete cell growth and passage systems to optimize your laboratory research. These proprietary media were created to generate the healthiest cells for your assessment. Use of other media may affect results.

These media can be procured separately or as a bundle at www.cell-systems.com.

Preparation for Freezing Cells:

1. Place PRG1 & 2 into the warm water bath and allow to reach 37°C. Chill the PRG3 and Cell Freezing Medium to ~0°C in the ice water bath. Chill the sterile, labeled cryogenic vials in a Styrofoam (or otherwise insulated) tray in -20°C.
2. Spray down the outside of all tools/instruments (bottles, tubes, racks, etc.) with the ethanol solution before bringing them under the hood. Make sure all work surfaces are disinfected. Spray the outside of your gloves prior to work under the hood.

Procedure for Freezing Cells:

1. Remove growth media from flask by pouring into waste receptacle or by aspiration.
2. Wash the culture by pipetting 7mL of pre-warmed PRG1 into the flask and spreading across the entire growth surface.
3. Remove PRG1 from flask by pouring into waste receptacle or by aspiration.

4. Pipette 7mL of pre-warmed PRG2 into the flask and spreading across the entire growth surface and immediately move to a microscope for observation.
5. As soon as >90% of the cells in culture have rounded up, quickly return to the hood and add 7mL of chilled PRG3 to the flask, ensuring that the entire growth surface has been covered.
6. Pipette the cell suspension up and allow to flow down over the growth surface twice, to wash the surface and collect any viable cells still adhering to the growth surface.
7. Transfer the entire cell suspension volume into a 15ml conical tube.
8. Centrifuge at 900 g and 4^{o}C for 10 min.
9. When the centrifugation has finished, immediately remove the conical tube, disinfect with ethanol solution and move under the hood.
10. Carefully remove medium supernatant from the tube by aspirating or decanting (pouring), ensuring that you do not disturb the cell pellet at the bottom of the tube.
11. Re-suspend the cells in the desired amount of ice-cold Cell Freezing Medium in order to obtain 0.5-2.0x10^{6} cells/ml. Gently draw the cell suspension up and down in order to mix and break up any cell aggregates.
12. Aliquot the cell suspension to the chilled, labeled, sterile cryogenic vials (1ml per vial).
13. Reduce the temperature at 1^{o}C per minute until temperature is <-70^{o}C. We recommend placing the vials in a Nalgene Cryo 1^{o}C Freezing Container filled with chilled isopropyl alcohol, and placing this Freezing Container into a -80^{o}C freezer for 90 minutes. Subsequently, move cryogenic vials to storage under liquid nitrogen.