Cell Freezing Medium™ (4Z0-705)

Product Description: Cell Freezing Medium provides the optimal environment for the freeze/thaw cycle of cell cultures. This is a specialized medium. When used in conjunction with Cell Systems Passage Reagent Group™, it provides the optimal environment for the freeze/thaw cycle of cell cultures and assists in minimizing cellular damage during the process.

Cell Systems media and reagents are sterile, made with WFI and all components are cGMP and ISO Compliant.

Components: Cell Systems Cell Freezing Medium (1X) 50mL. Contains Conditioned Cell Systems Media.

Storage: Store refrigerated (+2 - 8°C). Once opened, shelf life is 30 days at +2 - 8°C.

Product Use: 4Z0-705 is for laboratory research only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Shipping: All medium and reagents shipped at ambient temperature.

Instructions for Use: Make sure all work surfaces are disinfected. Spray the outside of your gloves prior to work under the hood.

Spray the outside of all tools/instruments (bottles, tubes, racks, etc.) with ethanol solution before bringing them under the hood.

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. Remove growth media from flask by pouring into waste receptacle or by aspiration.
3. Wash the culture by pipetting 7mL of pre-warmed PRG1 into the flask and spreading across the entire growth surface.
4. Remove PRG1 from flask by pouring into waste receptacle or by aspiration.
5. Pipette 7mL of pre-warmed PRG2 into the flask and spreading across the entire growth surface and immediately move to a microscope for observation.
6. 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.
7. 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.
8. Transfer the entire cell suspension into a 15mL conical tube.
9. Centrifuge at 900 g and 4°C for 10 min.
10. When the centrifugation has finished, immediately remove the conical tube, disinfect with ethanol solution and move under the hood.
11. 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.
12. 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.
13. Aliquot the cell suspension to the chilled, labeled, sterile cryogenic vials (1mL per vial).
14. Reduce the temperature at 1°C per minute until temperature is <-70°C. We recommend placing the vials in a Nalgene Cryo 1°C Freezing Container filled with chilled isopropyl alcohol and placing this Freezing Container into a -80°C freezer for 90 minutes. Subsequently, move cryogenic vials to storage under liquid nitrogen.