



Cryopreservation protocol. CleanCut GS CHO.

- 1.1. This procedure is only performed by qualified staff members.
- 1.2. Prepare BSC for cell culture work
- 1.3. Warm the complete medium
 - 1.3.1. Place complete CDForti CHO medium bottle into the 37°C water bath.
 - 1.3.2. When medium is warmed, remove bottle from water bath.
 - 1.3.3. Dry medium bottle completely with clean, dry paper towel.
 - 1.3.4. Spray and wipe medium bottle with 70% ethanol.
 - 1.3.5. Place medium bottle into the BSC.
- 1.4. Determine number of freezing vials
 - 1.4.1. Spray and wipe a bag of sterile 50mL tubes.
 - 1.4.2. Add the bag to the BSC.
 - 1.4.3. Open the bag in the BSC and remove one 50mL tube; place the tube into a tube rack.
 - 1.4.4. Use a serological pipette to transfer target CHO cell suspension from flask to 50mL tube.
 - 1.4.5. Gently resuspend the cells with a serological pipette 6-8 times.
 - 1.4.6. Quickly get a cell count using the automatic cell counter.
 - 1.4.7. Record the cell concentration, viability, and total cell number.
 - 1.4.8. Use the recorded data to determine how many vials to freeze – CHO suspension cells are typically frozen at 10×10^6 cells per vial.
 - 1.4.9. Label to each cryo-tube.
- 1.5. Freezing CHO Cells
 - 1.5.1. Centrifuge the 50mL tube of cell suspension at 125g for 7 minutes.
 - 1.5.2. Spray and wipe the tube with 70% ethanol before returning it to the BSC.
 - 1.5.3. Uncap the 50mL tube and connect a sterile Pasteur pipette to the BSC aspirator system.
 - 1.5.4. Carefully aspirate all supernatant from the cell pellet.
 - 1.5.5. Resuspend the pellet in 1mL of freezing medium per vial.

Example: 50×10^6 cells = 5 vials = 5mL freezing medium

Freezing medium: CryoStorCS10(Millipore Sigma, cat# C2874)

- 1.5.6. Aliquot the resuspended cells into the pre-labeled cryo-tubes; aliquot 1mL cell suspension per tube.
- 1.5.7. Cap the cryo-tubes and quickly transfer them to a Corning CoolCell container.
- 1.5.8. Immediately close the CoolCell and transfer to -80°C.
- 1.6. Transfer cells to liquid nitrogen storage (K-Series)
 - 1.6.1. Leave the cells in the CoolCell at -80°C overnight to slow freeze.
 - 1.6.2. The next business day, remove the CoolCell from the -80°C freezer and transfer the frozen cells from CoolCell in the liquid nitrogen storage box.

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