



Thawing protocol for 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 culture medium
 - 1.3.1. Place complete 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. Prepare thawing materials
 - 1.4.1. Spray and wipe a bag of sterile 15mL tubes with 70% ethanol.
 - 1.4.2. Add the bag to the BSC.
 - 1.4.3. Open the bag in the BSC and remove one 15mL tube; place the tube into a tube rack.
 - 1.4.4. Use a serological pipette to transfer 9mL of complete CHO medium to the 15mL tube.
- 1.5. Removing cells from liquid nitrogen
- 1.6. Thawing CHO suspension cells
 - 1.6.1. Carefully submerge the lower half of the frozen cell vial into the 37°C water bath. Do not submerge the cap.
 - 1.6.2. Rapidly thaw the frozen cells in the water bath with gentle rotation until only a small ice pellet remains – this takes about 2 minutes.
 - 1.6.3. Remove the thawed cells from the water bath.
 - 1.6.4. Dry the cell vial with a clean, dry paper towel.
 - 1.6.5. Spray and wipe the cell vial with 70% ethanol.
 - 1.6.6. Transfer thawed vial to the BSC.
 - 1.6.7. Use a 1mL pipette to gently transfer the thawed cells to the 15mL tube with 9mL prepared medium.
 - 1.6.8. Use some of the 9mL medium to rinse the cell vial – add the rinse back to the 15mL tube.

- 1.6.9. Gently resuspend the thawed cells with a serological pipette 6-8 times.
- 1.6.10. Quickly get a cell count using the automatic cell counter
- 1.6.11. Record the cell concentration, viability, and total cell number.
- 1.6.12. Centrifuge the cells at 125g for 7 minutes.
- 1.6.13. Remove the cells from the centrifuge; spray and wipe the tube with 70% ethanol.
- 1.6.14. Return the cells to the BSC.
- 1.6.15. Fully aspirate the supernatant from the cell pellet.
- 1.6.16. Resuspend the cell pellet with fresh medium and transfer cells into an E-125 flask.
- 1.6.17. Add media to the total volume in the E-125 flask so the final concentration of cells is 0.3×10^6 per mL.
- 1.6.18. Place the E-125 on a shaker located in a cell culture incubator.
- 1.6.19. Incubator is set at 37°C and 8% CO₂. Shaker is set at 125rpm.

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