CleanCut GS CHO Cryopreservation SOP

SOP004 | Version: 0E01 | Effective Date: 08/20/2026

Purpose

This SOP outlines Demeetra's standard method for cryopreservation of CleanCut GS CHO cell lines in CryoStor cell cryopreservation media (CS10).

Required Materials

Product or Reagent	Vendor	Catalog No.
CryoStor® cell cryopreservation media (CS10)	Millipore Sigma	C2874-100ML
Nalgene System 100 Cryogenic Tube externally threaded, 1.5 mL	Thermo Sci	5000-1020
Corning® CoolCell® LX, Cell Freezing Container, for 12 x 1 mL or 2 mL Cryogenic Vials	Corning	432001

Protocol

1. Culture CleanCut GS CHO cells to a cell density between 1-3x10^6 cells/mL.
2. Using a serological pipet, transfer the cells to a 50mL conical tube and gently mix the cells 6-8 times.
3. Quickly measure the cell density using an automatic cell counter. Record the cell concentration, viability, and total cell number.
4. Use the recorded data to determine how many vials to freeze – CHO suspension cells are typically frozen at 10x10^6 cells per vial.
5. Label to each cryo-tube.
6. Centrifuge the 50mL tube of cell suspension at 125 RCF for 7 minutes.
7. Spray and wipe the tube with 70% ethanol before returning it to the BSC.
8. Carefully aspirate the cell supernatant
9. Resuspend the pellet in 1mL of cold (2 - 8°C) CryoStor CS10 media per vial by carefully mixing 6-8 times.
   Example: 50x10^6 cells = 5 vials = 5mL freezing medium.
10. Aliquot the resuspended cells into the pre-labeled cryo-tubes; aliquot 1mL cell suspension per tube.

11. Cap the cryo-tubes and quickly transfer them to a Corning CoolCell container.
12. Immediately close the CoolCell and transfer to -80°C.
13. Leave the cells in the CoolCell at -80°C overnight to slow freeze.
14. The next business day, remove the CoolCell from the -80°C freezer and transfer the frozen cells from the CoolCell to storage in liquid nitrogen vapor phase.

❖ *Note: do not store cells at -80°C for longer than 72 hours.*