CleanCut GS CHO Thawing SOP

SOP001 | Version: 1E01 | Effective Date: 08/20/2026

Purpose

This SOP outlines Demeetra's standard method for thawing CleanCut GS CHO cell lines. Demeetra cell lines are frozen in CryoStor® cell cryopreservation media (CS10) and should be stored in liquid nitrogen vapor phase.

Required Materials

Product or Reagent	Vendor	Catalog No.
Fisherbrand™ Disposable PES Filter Units	Fisher Sci	FB12566506
GlutaMAX™ Supplement	Gibco	35050061
CD FortiCHO™ Medium	Gibco	A1148301
Anti-Clumping Agent	Gibco	0010057AE
HyClone™ Penicillin-Streptomycin 100X solution	Cytiva	SV30010
125 mL Polycarbonate Erlenmeyer Flask (E-125)	Corning	431143

Media Preparation

Complete CHO Medium (1L)

Add the following components to a 0.2 µm aPES 1L filter unit. Filter by suction once all components are added.

- 40mL GlutaMAX™ Supplement (final conc – 8mM)
- 5mL Penicillin-Streptomycin (*Note: this component is optional*; final conc – 0.5%)
- 1mL Anti-Clumping Agent (final conc – 1:1000)
- Bring total volume to 1000mL with CD FortiCHO Medium

❖ *Note: Complete CHO Medium is good for 2 weeks stored at 4°C. CleanCut GS CHO cell lines are adapted to grow in this formulation and we do not recommend changing the components. GlutaMAX can be substituted for L-Glutamine, but this may decrease the medium shelf life. Omitting Anti-Clumping Agent may result in decreased cell viability or increased cell recovery time or doubling times.*

Standard CleanCut GS CHO Cell Incubation Conditions

Temperature: 37°C

CO_2 level: 8%

Shaking:

- Well plates – Do not shake
- T-25 Flasks – 90 RPM, 19mm Orbit
- E-125 Flasks – 125 RPM, 19mm Orbit

Thawing Protocol

Before thawing:

1. Pre-warm Complete CHO Medium bottle in a 37°C water bath.
2. Prepare biosafety cabinet (BSC) for cell culture work by turning on UV for 20min, followed by spraying and wiping with 70% ethanol.
3. Dry the Complete CHO Medium bottle completely with clean, dry paper towel, spray and wipe with 70% ethanol, and place into the BSC.
4. Use a serological pipette to transfer 9mL of complete CHO medium to a 15mL tube.

Thawing CHO suspension cells:

5. Remove cells from liquid nitrogen storage and ensure vials go directly to warming.

❖ *Note: If thawing multiple vails, pour liquid nitrogen into a foam container and keep the cells floating in a liquid nitrogen bath until ready to thaw.*

6. Carefully submerge the lower half of the frozen cell vial into a 37°C water bath. Do not submerge the cap.
7. Rapidly thaw the frozen cells in the water bath with gentle rotation until only a small ice pellet remains – this takes about 2 minutes.
8. Dry the cell vial with a clean, dry paper towel.
9. Spray and wipe the cell vial with 70% ethanol.
10. Transfer thawed vial to the BSC.
11. Use a 1mL pipette to gently transfer the thawed cells to the 15mL tube with 9mL prepared medium.
12. Use some of the medium from the same 15mL tube to rinse the cell vial – add the rinse back to the 15mL tube.
13. Gently resuspend the thawed cells with a serological pipette 6-8 times.
14. Quickly get a cell count using an automatic cell counter and record the cell concentration, viability, and total cell number.

15. Centrifuge the cells at 125g for 7 minutes.
16. Remove the cells from the centrifuge; spray and wipe the tube with 70% ethanol.
17. Return the cells to the BSC.
18. Fully aspirate the supernatant from the cell pellet.
19. Resuspend the cell pellet with fresh medium and transfer cells into an E-125 flask.
20. Add media to the total volume in the E-125 flask so the final concentration of cells is $0.3x10^6$ cells per mL.
21. Place the E-125 on a shaker located in a cell culture incubator.
22. Incubator is set at 37°C and 8% CO2. Shaker is set at 125 RPM.