Transfection Recovery and GS Selection SOP

SOP002 | Version: 1E01 | Effective Date: 08/20/2026

Purpose

This SOP outlines Demeetra's recommended workflow for growth and glutamine synthetase (GS) selection of CleanCut GS CHO Cell lines following transfection in either 24-well or 6-well plate formats.

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
Nunc™ Non-Treated 24-Well Plate	Thermo Sci	12-566-82
Nunc™ Non-Treated 6-Well Plate	Thermo Sci	12-566-80
25 cm^2 Rectangular Canted Neck Not Treated Cell Culture Flask (T-25)	Corning	431463
125 mL Polycarbonate Erlenmeyer Flask (E-125)	Corning	431143

Media Preparation

Transfection Growth 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)
- 1mL Anti-Clumping Agent (final conc – 1:1000)
- Bring total volume to 1000mL with CD FortiCHO Medium

GS Selection Medium (1L)

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

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

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

Protocols

24-Well Plate Transfection Recovery Protocol

Start this protocol after transfected cells have been prepared with a cell count of 0.2-0.25x10^6 cells per well in a total of 500µl of Transfection Growth Medium.

1. Following transfection, incubate cells under Standard CleanCut GS CHO Cell Incubation Conditions for 24hr without disturbing the cells. Do not shake the plate.

❖ ***Critical Checkpoint:*** *Use a microscope to observe cells 24-48 hours post-transfection. Check for fluorescence in control cells. You may see some cell debris and cell clumping. This is normal. Clumping typically resolves after transferring to a shaking flask.*

2. At 48hr post-transfection add 600µl of Transfection Growth Medium, gently mix the cells, and split the cell volume into 2 wells of the 24-well plate such that each well has approximately 500µl. Return the plate to the incubator without shaking.
3. At 72hr post-transfection, transfer cells to a T-25 shake flask:
    a. Add 2ml of Transfection Growth Medium to a T-25 flask in the vertical position.
    b. Gently transfer the contents of both wells to the T-25 flask.
    c. With 1ml of fresh Transfection Growth Medium, wash the two wells of the 24-well plate to recover any left-behind cells and add this to the flask as well.
    d. Place flask horizontally on the shaker in the incubator and shake at 90 RPM.
4. At 96hr post-transfection, gently mix the cells by pipetting and measure cell viability and viable cell density (VCD). If viability is ≥85% and VCD is >1.0 × 10^6 cells/mL, proceed to GS Selection. Otherwise, continue growing cells until these conditions are met before continuing to GS Selection. Add or replace the cell media with fresh Transfection Growth Medium every 3-4 days. Do not allow total volume in the T-25 flask to exceed 6mL.

❖ ***Critical Checkpoint:*** *Exercise caution if viability is below 70% on day 4. This may indicate problems in the transfection or culturing processes. We recommend using post-transfection viability as a critical checkpoint for evaluating transfection success and predicting downstream success.*

6-Well Plate Transfection Recovery Protocol

Start this protocol after transfected cells have been prepared with a cell count of 1.0-2.0x10^6 cells per well in a total of 2mL of Transfection Growth Medium.

1. Following transfection, incubate cells under Standard CleanCut GS CHO Cell Incubation Conditions for 24hr without disturbing the cells. Do not shake the plate.

❖ ***Critical Checkpoint:*** *Use a microscope to observe cells 24-48 hours post-transfection. Check for fluorescence in control cells. You may see some cell debris and cell clumping. This is normal. Clumping typically resolves after transferring to a shaking flask.*

2. At 48hr post-transfection, transfer cells to a T-25 shake flask:
    a. Add 1ml of Transfection Growth Medium to a T-25 flask in the vertical position.
    b. Gently transfer the 2mL total of cells to the T-25 flask.
    c. With 1ml of fresh Transfection Growth Medium, wash the well of the 6-well plate to recover any left-behind cells and add this to the flask as well.
    d. Place flask horizontally on the shaker in the incubator and shake at 90 RPM.

❖ ***Note:*** *For quicker scale-up, the total volume from multiple wells can be combined and transferred to an E-125 shake flask instead of a T-25. For instance, combine 4mL of cells with 4mL of Transfection Growth Medium and add to an E-125 flask; shake at 125 RPM.*

3. At 72hr post-transfection, gently mix the cells by pipetting and measure cell viability and viable cell density (VCD). If viability is ≥85% and VCD is >1.0 × 10^6 cells/mL, proceed to the **GS Selection Protocol**. Otherwise, continue growing cells until these conditions are met. Add or replace the cell media with fresh Transfection Growth Medium every 3-4 days. Do not allow total volume in the T-25 flask to exceed 6mL.

❖ ***Critical Checkpoint:*** *Exercise caution if viability is below 70% on day 4. This may indicate problems in the transfection or culturing processes. We recommend using post-transfection viability as a critical checkpoint for evaluating transfection success and predicting downstream success.*

GS Selection Protocol

1. After cells have reached a viability of at least 85% with a VCD greater than $1.0x10^6$ cells/mL in the T-25 shake flask, centrifuge the cells at 125 RCF for 7min.
2. Aspirate the old media and replace with GS Selection Medium to a seeding density of $1.0x10^6$ cells/mL.
3. Transfer 4-6 mL of cells in GS Selection Medium to a new T-25 flask and shake at 90 RPM. Alternatively, transfer 7-30mL of cells in GS Selection Medium to an E-125 flask and shake at 125 RPM.
4. Prepare a mock/un-transfected sample of cells in GS Selection Medium along with your transfected samples as a control for the GS selection.
5. Measure cell viability and VCD every 2-3 days to monitor the selection.

❖ ***Critical Checkpoint:*** *Expect the viability of the mock to drop drastically by day 4 on selection and to be totally dead by day 8-14. During GS selection of CleanCut GS CHO cell lines transfected with vectors containing Demeetra's standard glutamine synthetase gene setup, it is normal to see some drop in viability. However, it is also common for transfections with high transfection and integration efficiencies to yield cell populations which maintain viabilities higher than 85% over the course of the whole selection process. As long as a normal drop in viability is seen in the mock culture, do not be alarmed if a significant drop in viability is not observed in transfected cultures. Conversely, exercise caution if viability drops by more than 50% in a transfected culture, as this may be an indication of poor transfection or integration efficiencies. We recommend using viability on GS selection as a critical checkpoint for evaluating transfection success and predicting downstream success.*

6. After 72hr of selection, add or replace the cell media with fresh GS Selection Medium based on total cell count, scaling up or passaging cells if necessary, according to the chart below:

Flask	Total Cell Count ($x10^6$ cells)	Action
T-25	<3	Centrifuge, remove all but 3mL to concentrate cells, resuspend. Do not add medium.
T-25	3-6	Centrifuge, remove half of media and replace with fresh GS Selection Medium, resuspend.
T-25	>6	Seed at $0.5x10^6$ cells/mL, 7-30mL in E-125.
E-125	<6	Centrifuge, remove all but 2mL, resuspend at $1x10^6$ cells/mL, 3-6mL in T-25.
E-125	>6	Seed at $0.5x10^6$ cells/mL, 7-30mL in E-125.

7. Passage cells to 0.5x10^6 cells/mL in GS Selection Medium every 2-3 days.

❖ ***Note:*** *Once total cell count reaches at least 15x10^6 cells, passage into a total of 30mL. If cell viability stabilizes above , begin passaging at the standard 0.3x10^6 cells/mL, 30mL in GS Selection Medium every 2-3 days.*

8. Carry out cell selection for a total of 14 days.