CleanCut GS CHO Single-cell Cloning SOP

SOP003 | Version: 1E01 | Effective Date: 08/20/2026

Purpose

This SOP outlines a general workflow for the preparation and handling of CleanCut GS CHO cells before and after single-cell cloning. It is intended to support a variety of single-cell cloning methods and platforms, including limiting dilution cloning and cell-dispenser instrument-based workflows. Detailed procedures for performing specific single-cell cloning methods are outside the scope of this SOP.

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
Solentim® InstiGRO CHO PLUS	Advanced Instruments	RS-1225
Nunc™ Non-Treated 24-Well Plate	Thermo Sci	12-566-82
25 cm^2 Rectangular Canted Neck Not Treated Cell Culture Flask (T-25)	Corning	431463

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

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

❖ *Note: For cells that underwent GS selection, we recommend continuing to use GS Selection Medium for single-cell cloning.*

Single-Cell Cloning Medium (50mL)

Combine InstiGRO CHO PLUS with either Complete CHO Medium or GS Selection Medium to a final concentration of 5%.

- Add 2.5mL InstiGRO CHO to the bottom of a 50mL conical (final conc – 5%)
- Add 47.5mL media.
- Vortex to mix.

❖ *Note: From this part of the protocol forward, any mention of "media" that is not Single-Cell Cloning Medium refers to either Complete CHO Medium or GS Selection 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

Protocol

1. One day prior to single-cell seeding, passage cells to $1x10^6$ cells/mL in 30mL in an E-125.
2. On the day of seeding, use the single-cell seeding method of choice to seed 1 cell in 200µL of Single-Cell Cloning Medium per well into a 96-well plate.

3. For monoclonality confirmation, image the wells within 5hr of seeding.
4. Allow cells to incubate for 14 days, imaging the wells again on day 7 to monitor growth.
5. On Day 14, image the cells again and feed with 60µL of fresh media.
6. On Day 15, consolidate clones with verified monoclonality and confluency of at least 33% to a new 96-well plate. To do this, gently mix each well and transfer the entire volume to the new plate.
7. On day 17, transfer each clone from the 96-well plate to a low-attachment 24-well plate. To do so, add 400µL media to each well of the new plate and transfer the full volume of cells.

❖ *Note: Alternatively, add 200µL of fresh media to the 96-well plate, mix, and transfer 200µL of cells to a new 96-well plate. Use the cells from one plate for DNA extraction for genotyping and transfer corresponding clones with correct genotype to at 24-well plate 24-72hr later.*

8. When total cell count reaches at least $1x10^6$ cells, transfer cells into a T-25 with 5mL media. Shake at 90 RPM.
9. When Cell number reaches at least $3x10^6$ cells, transfer into E-125 shaker flask 0.3x10^6/mL in 10-30 mL media on shaker at 125 RPM.