



Single Cell Cloning Protocol. CleanCut GS CHO

1. Thaw or reseed cells and place in incubator at 37° C, 8% CO₂ one day prior to cloning; seed at 1×10^6 cells/mL in E125.
2. On the day of seeding, seed 1 cell per well using limited dilution or VIPs into a 96 well plate at 200 uL media per well. Use the following Medium: CDForti CHO + 5% CHO InstiGRO+
3. Allow cells to incubate for 2 weeks, monitoring via scan or microscope for cell growth.
4. On Day 14, allow cells to settle to the bottom of plate and collect 100 uL supernatant and transfer into a separate 96 well plate. NOTE: Supernatant used for Valita Cell IgG Assay for titer in step 6.
5. Add 160uL fresh media back to plate with cells.
6. Perform Valita assay on all wells or wells of interest to assess titer.
7. On day 17 transfer verified clones from 96 well plate into 24 well low attachment plate.
8. When cell number reaches 1×10^6 cells, transfer cells into non treated T25 with 5 mL media. Shake at 90 RPM in incubator at 37° C, 8% CO₂.
9. When Cell number reaches 9×10^6 , transfer into E125 shaker flask 0.3×10^6 /mL in 30 mL media on shaker at 125 RPM.