ClonaCell™-CHO CD Liquid Medium

Chemically defined, animal component-free, serum-free, protein-free, glutamine-free liquid medium for culturing CHO cells

Catalog # 03817 500 mL

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

ClonaCell™-CHO CD Liquid Medium is a general purpose liquid medium for culture of suspension-adapted CHO cells. This chemically defined liquid medium is protein-free and animal component-free. It does not contain L-glutamine, selection agents, antibiotics, or phenol red. ClonaCell™-CHO CD Liquid Medium is compatible with a variety of selection systems, including dihydrofolate reductase (DHFR) and glutamine synthetase (GS).

- Supports expansion of CHO cells for protein expression and other applications in a chemically defined, protein- and animal component-free medium
- Compatible with ClonaCell™-CHO CD Medium (Catalog #03815), ClonaCell™-CHO ACF Supplement (Catalog #03820), ClonaCell™-TCS Medium (Catalog #03814), and ClonaCell™ FLEX (Catalog #03818)

Properties

Storage: Store at 2 - 8°C. Protect from light.

Shelf Life: Stable until expiry date (EXP) on label.

Contains:
- Kolliphor P188
- Other ingredients

Protect product from light.

Handling / Directions For Use

All procedures should be carried out using aseptic technique in a certified biosafety cabinet.
Warm media to room temperature (15 - 25°C) or 37°C prior to use.

ClonaCell™-CHO CD Liquid Medium does not contain L-glutamine and should be supplemented with 4 - 8 mM L-Glutamine (Catalog #07100) if required. Additional supplements, antibiotics, or selective agents may also be added to the medium if needed. If antibiotics are to be used for selection, an appropriate concentration should be determined for CHO cells cultured in ClonaCell™-CHO CD Liquid Medium prior to use.

DIRECT ADAPTATION OF CHO CELLS TO ClonaCell™-CHO CD LIQUID MEDIUM

Most cell lines can be transferred directly into ClonaCell™-CHO CD Liquid Medium.

1. Determine the cell concentration and viability of culture. Cells should be in logarithmic growth phase with a viability of > 90% prior to adapting them directly to growth in ClonaCell™-CHO CD Liquid Medium.
2. Seed flasks at 3 x 10^5 viable cells/mL.
3. Incubate at 37°C in 5% CO_2 and ≥ 95% humidity.
4. Passage every 3 - 4 days at seeding densities of 1 - 3 x 10^5 viable cells/mL.
   NOTE: Passage for a minimum of 3 passages where viability is > 90% and cell growth is good prior to cryopreserving CHO cells adapted to ClonaCell™-CHO CD Liquid Medium.

SEQUENTIAL ADAPTATION OF CHO CELLS TO ClonaCell™-CHO CD LIQUID MEDIUM

Sequential adaptation of CHO cells may be required if direct adaptation proves problematic.

1. Seed flasks at 3 x 10^5 cells/mL in a mixture of the original growth medium and ClonaCell™-CHO CD Liquid Medium at a ratio of 3:1. Incubate at 37°C in 5% CO_2 and ≥ 95% humidity for 3 - 4 days.
2. Determine the cell concentration and viability of the culture. If viability is > 90% and the cell concentration is > 1 x 10^6 cells/mL, proceed to the next step. If viability is low and growth is slow, maintain or passage cells in the 3:1 medium ratio until viability and growth increases.

3. Change medium to a 1:1 ratio of the original medium and ClonaCell™-CHO CD Liquid Medium. Incubate at 37°C in 5% CO_2 and ≥ 95% humidity for 3 - 4 days.
4. Determine the cell concentration and viability of the culture. If viability is > 90% and the cell concentration is > 1 x 10^6 cells/mL, proceed to the next step. If viability is low and growth is slow, maintain or passage cells in the 1:1 medium ratio until viability and growth increases.
5. Change medium to a 1:3 ratio of the original medium and ClonaCell™-CHO CD Liquid Medium. Incubate at 37°C in 5% CO_2 and ≥ 95% humidity for 3 - 4 days.
6. Determine the cell concentration and viability of the culture. If viability is > 90% and the cell concentration is > 1 x 10^6 cells/mL, proceed to the next step. If viability is low and growth is slow, maintain or passage cells in the 1:3 medium ratio until viability and growth increases.
7. Change medium to 100% ClonaCell™-CHO CD Liquid Medium. Incubate at 37°C in 5% CO_2 and ≥ 95% humidity for 3 - 4 days.
8. Determine the cell concentration and viability of the culture. If viability is > 90% and the cell concentration is > 1 x 10^6 cells/mL, proceed to the next step. If viability is low and growth is slow, maintain or passage cells in 100% ClonaCell™-CHO CD Liquid Medium until viability and growth increases.
9. Passage every 3 - 4 days at seeding densities of 1 - 3 x 10^5 viable cells/mL. Passage for a minimum of 3 passages where viability is > 90% and good cell growth is observed prior to cryopreserving CHO cells adapted to ClonaCell™-CHO CD Liquid Medium.

CRYOPRESERVATION

Cryopreserve cells at 0.5 - 1 x 10^7 cells/mL.

1. Prepare cryopreservation medium (ClonaCell™-CHO CD Liquid Medium containing 7.5% DMSO) and place on ice.
   NOTE: A 1:1 mixture of conditioned medium and fresh ClonaCell™-CHO CD Liquid Medium, to which DMSO has been added to 7.5%, may also be used.
2. Centrifuge cells at 300 x g for 5 - 10 minutes.
3. Aspirate supernatant and resuspend cells in cold cryopreservation medium.
4. Aliquot into sterile cryovials.
5. Place into freezing containers and freeze as per manufacturer's instructions.
6. Remove cryovials from freezing containers and store in liquid nitrogen.

THAWING CRYOVIALS

1. Remove cryovials from liquid nitrogen.
2. Thaw in a 37°C water bath.
3. Slowly add 1 mL of warm ClonaCell™-CHO CD Liquid Medium dropwise to thawed cells.
4. Gently transfer cells to a 14 mL tube.
5. Slowly add 5 - 10 mL of medium to cells with gentle swirling.
6. Centrifuge cells at 300 x g for 5 - 10 minutes.
7. Remove supernatant. Resuspend in medium and seed at 3 x 10^5 cells/mL.

EXPANSION OF COLONIES PICKED FROM SEMI-SOLID MEDIUM

CHO cell colonies subcloned and/or selected in semi-solid ClonaCell™-CHO CD Medium (Catalog #03815) may be picked and transferred to ClonaCell™-CHO CD Liquid Medium for expansion.

1. Dispense 200 µL of ClonaCell™-CHO CD Liquid Medium into each well of a 96-well plate.
2. Pick colonies from semi-solid medium. Deposit each colony into a well of the 96-well plate containing medium.
3. Pipette up and down to disperse cells.
4. Incubate at 37°C in 5% CO_2 and ≥ 95% humidity.

For further information, refer to the Product Information Sheet for ClonaCell™-CHO CD Medium (Document #29996), available at www.stemcell.com or contact us to request a copy.

References

Wognum B & Lee T. (2013) Simultaneous cloning and selection of hybridomas and transfected cell lines in semisolid media. Methods Mol Biol 946: 133–49.

Young ARJ et al. (2013) Cell senescence as both a dynamic and a static phenotype. Methods Mol Biol 965: 1–13.