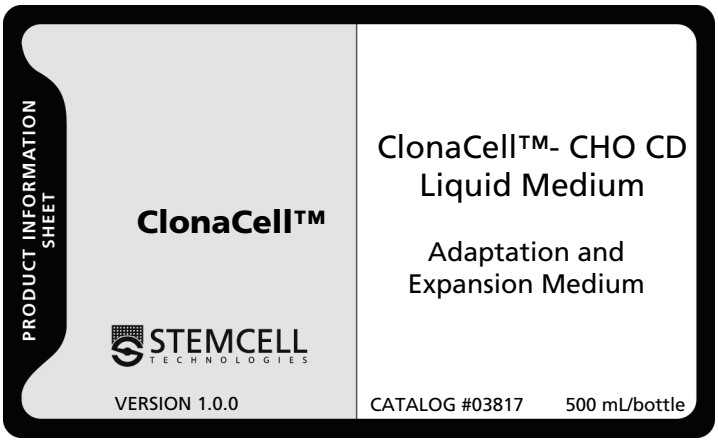
PRODUCT DESCRIPTION

ClonaCell™-CHO CD Liquid Medium is a chemically-defined, serum-free, protein-free, animal component-free liquid medium. This medium is used for the culture of Chinese Hamster Ovary (CHO) cells. This medium can also be used for the expansion of clones that have been selected and cloned in ClonaCell™-CHO CD semi-solid medium.

ClonaCell™-CHO CD Liquid Medium contains Pluronic F68. It does not contain L-glutamine, antibiotics or phenol red.



FORMAT

03817 ClonaCell™-CHO CD Liquid Medium 500 mL

Contains: Serum-free, protein-free, animal component-free, chemically-defined CHO culture medium.
Does not contain: *Hypoxanthine, aminopterin, thymidine, glutamine, or any other selective agents or antibiotics.*

STABILITY AND STORAGE

Product stable at 2 - 8°C until expiry date as indicated on label.

Protect from light.

DIRECTIONS FOR USE

All procedures should be carried out using sterile techniques in a certified biosafety cabinet. Pre-warm medium to room temperature or 37°C prior to use.

ClonaCell™-CHO CD Liquid Medium is formulated without L-glutamine, supplement with 4-8mM L-glutamine if needed. Additional supplements, antibiotics or selective agents may also be added to the medium if necessary. (Antibiotic selection levels should be determined for CHO cells in ClonaCell™-CHO CD Liquid Medium prior to use.)

The viability of cells should be greater than 90% and the cells should be in logarithmic growth phase prior to adapting directly or sequentially in ClonaCell™-CHO CD Liquid Medium.

Direct Adaptation of CHO cells to ClonaCell™-CHO CD Liquid Medium

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

1. Determine the cell concentration and viability of culture.
2. Seed flasks at 3 x 10^5 viable cells/mL.
3. Incubate at 37°C in a humidified, 5% CO_2 incubator.
4. 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 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

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

1. Culture cells at 3x10^5 cells/mL in a mixture of the original growth medium and ClonaCell™-CHO CD Liquid Medium at a ratio of 3:1.
2. 3-4 days later, determine the cell concentration and viability of the culture. If viability is >90% and cell concentration is >1x10^6 cells/mL, proceed to the next step. If viability is low and growth is slow, maintain or passage cells in the current media ratio until viability and growth increases.

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3. Culture cells in a 1:1 ratio of the original medium and ClonaCell™-CHO CD Liquid Medium.
4. 3-4 days later, examine cells as in Step 2.
5. Culture cells in a 1:3 ratio of the original medium and ClonaCell™-CHO CD Liquid Medium.
6. Examine cells as in Step 2.
7. Culture cells in 100% ClonaCell™-CHO CD Liquid Medium. Examine cells as in Step 2.
8. 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 cell growth is good prior to cryopreserving CHO cells adapted to ClonaCell™-CHO CD Liquid Medium.

Cryopreservation

Cryopreserve cells at 5-10 x 10^6 cells/mL in ClonaCell™-CHO CD Liquid Medium containing 7.5% DMSO.

1. Prepare cryopreservation medium of ClonaCell™-CHO CD Liquid Medium containing 7.5% DMSO and place on ice. (Note: 50% conditioned medium, 50% fresh ClonaCell™-CHO CD Liquid Medium plus 7.5% DMSO may also be used.)
2. Centrifuge cells at 300 x g for 5-10min.
3. Aspirate off supernatant. Resuspend 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

1. Remove cryovials from liquid nitrogen.
2. Thaw in a 37°C waterbath.
3. Slowly add 1mL of prewarmed ClonaCell™-CHO CD Liquid Medium dropwise to thawed cells.
4. Gently transfer cells to 14mL Falcon tube.
5. Slowly add 5-10mL of medium to cells with gentle swirling.
6. Centrifuge cells at 300 x g for 5-10min.
7. Remove supernatant. Resuspend in medium and seed culture at 3x10^5 cells/mL.

Expansion of colonies from semi-solid medium

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

1. Dispense 200µL 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 200µL medium.
3. Pipette up and down to disperse cells.
4. Culture at 37°C in a humidified, 5% CO_2 incubator.

Refer to the ClonaCell™-CHO CD Medium (Catalog # 03815) product information sheet for further details, available on our website at www.stemcell.com

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