Product Name:	**QuaCell® CHO CD02 Medium**
Cat. No.	**A12002**
Amount:	**10L, 100L, or customized**
Formulation:	**Powder**
Storage:	**2~8°C**
Validity period:	**24 months (Validity period on product packaging)**

Description

QuaCell® CHO CD02 Medium provides a rich environment for high density CHO cell growth. QuaCell® CHO CD02 is a serum-free, animal component-free, chemically defined medium developed for CHO suspension culture for expression of antibodies and protein products. CHO suspension cultures can be subculture directly into QuaCell® CHO CD02 Medium from serum-supplemented or serum-free medium with little or no adaptation. QuaCell® CHO CD02 Medium is formulated without hypoxanthine, thymidine and L-glutamine, suitable for DHFR, glutamine synthetase (GS System) screening system.

Components

L-glutamine	No
Glucose	6.0 g/L
Hypoxanthine & Thymidine	No
Phenol red	No
Sodium bicarbonate	No
Hydrolysate	No

Product Intended Use

Use aseptic technique when handling or supplementing this medium. This product is for research or for further manufacturing use.

CAUTION: Not for human or animal therapeutic use. Uses other than the intended use may be a violation of local law.

Safety information

Read the Material Safety Data Sheets (MSDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Preparation Instructions

- Preparation method

1. Add 90% of the final volume of water for injection to a suitable clean container and adjust the water temperature to 25 ~ 35 ℃.
2. Slowly add 17.38 g/L QuaCell® CHO CD02 medium powder, stir and mix for 10 minutes.
3. Slowly add 5 mol/L NaOH adjust the pH to 9.05 ± 0.10 and stir 30 minutes.
4. Slowly add 5 mol/L HCl adjust the pH to 7.00 ± 0.10 and stir 10minutes.
5. Slowly add 1.90 g/L $NaHCO_3$ and stir 10 minutes until dissolved.
6. Add 2.70 g/L NaCl stir 10 minutes until dissolved.
7. Use 5 mol/L HCl or NaOH to adjust the pH to 7.00 ± 0.10.
8. Add water for injection to the desired final volume, stir and mix well.
9. Sterilize by filtration using pore size 0.20μm filter.

- Use QuaCell® CHO CD02 medium under sterile condition.
- This product is formulated without L-glutamine, add L-glutamine as needed before use.
- Antibiotics are not recommended.
- The unused medium after opening should be sub package, Use sealing film to seal, In 2 ~ 8 ℃ avoid light preservation.

Condition of cell culture

Medium: complete QuaCell® CHO CD02 medium
Cell Line: CHO cells
Culture type: suspension
Culture container: shaker /TPP/ reactor
Temperature range: 37℃±0.5℃
Incubator air requirements: humidified with 5%~8% CO_2
Note: ensure proper air exchange and minimum exposure.

Cell recovery

1. Quickly thawing cryopreservation tube in 37 ℃ water (< 2 minutes) .
2. Transfer Cell liquid to 15 mL centrifuge tube. Add 10 mL of preheating QuaCell® CHO CD02 medium, centrifugate at 1,000 rpm for 5 min, discard the supernatant, using 5 mL QuaCell® CHO CD02 medium suspension, cell counting.
3. Use an automated cell counter or other counting instrument to count cells, pipette the cell fluid to a 125 mL shaker flask as needed for cell density, and add an appropriate amount of QuaCell® CHO CD02 medium to achieve the desired resuscitation density;the recommended resuscitation density is (3~5) × 10^5 cells/mL.
4. Culture in incubator contains 5% ~ 8% CO_2, 37℃ humidified air.
5. Cells should be cultured for 2 to 5 days after cell recovery and passed on in the middle stage of logarithmic growth. The recovered cells should be passed on at least three times before other experiments.

Cell passage advice

1. Use automatic cell counting apparatus or other counting

instruments to count the cells, and passage them according to the required density or in proportion. Recommended inoculation density is (3~5)×10^5 cells/mL.

2. Add the cells to a shaker flask containing QuaCell® CHO CD02 medium to achieve the desired seeding density of the cells.

3. Continue culture at 37 ℃, 5% ~ 8% CO_2 in the shake incubator, usually passage cells after 2 ~ 3 days.

Adaptation

CHO suspension cultures can be directly cultured into QuaCell® CHO CD02 medium from serum-supplemented or serum-free medium with little or no adaptation.

It is essential that the cell is in the middle of logarithmic growth and make sure the cell viability is beyond 90% before the initiation of the adaption process.

Directly culture

Transfer the suspension culture cells to QuaCell® CHO CD02 medium, as follows:

1. Centrifuge cell suspension at 1000rpm for 3 to 5 minutes. remove and discard the supernatant.
2. Suspension cells into preheat fully QuaCell® CHO CD02 medium in (3 ~ 5) X 10^5 cells/mL of living cells density and transfer to an appropriate culture flask.
3. Put culture flask back to the shake incubator and observe cell growth.

Note: if unsatisfactory cell growth is observed using procedure adaption as follows.

Procedure adaption

Steps of cell suspension culture:

1. Make cell density of (4 ~ 5) X 10^5 cells/mL during the adaptation process.
2. Gradual adjustment QuaCell® CHO CD02 medium with the original proportion of cell culture medium（25:75，50:50，75:25，90:10，then 100% QuaCell® CHO CD02 medium）. Cells can be passaged several times depending on the situation in each step.
3. After been passaged in 100% QuaCell® CHO CD02 medium a few times, the living cell density should beyond (1 ~ 2) X 10^6 cells/mL, The cell viability within 4 ~ 6 days was greater than or equal to 85%. In this phase, cells are considered suitable for QuaCell® CHO CD02 medium. In the final stage of adaptation, the cell density can be reduced to (2~3) X 10^5 cells/mL.

Cryopreservation

1. Prepare the required number of cells and the cells are in good condition.
2. Use an automatic cell counter or other counting instrument for cell counting, and calculate the volume required for cryopreservation medium according to the final cryopreservation density, and the recommended cryopreservation density is >1 × 10^7 cells/mL.
3. Centrifugate at 1,000 rpm for 5 min, discard the supernatant, and resuspend the cells with an appropriate amount of cryopreservation medium to cryopreservation density.
4. The cell suspension should be immediately repackaged into a cryopreservation tube according to the specifications
5. According to the standard procedure, cryopreservation in automatic or manual control freezing equipment. Transfer cells into the liquid nitrogen, stored under -130℃.

Note: after 24 hours of storage in liquid nitrogen, remove one to examine the viability and other indicators. See "cell recovery".

Fed-batch culture advice

- It is recommended to add feed according to the QuaCell® CHO Feed product profile.
- Day4 begins to measure the sugar concentration of the cells; If the cell growth rate is fast, Day3 starts to measure the sugar concentration of the cell, and the sugar value is lower than 3g/L to 6g/L; If there is a feeding operation on the same day, it is recommended to perform sugar testing 1 h after feeding.
- Day14 or when the cell viability is less than 70% is harvested, the expression volume and other data are analyzed.
- If the project already has a mature culture process, it is recommended to use the original process for trial. If the process is in development stage, it is recommended to use the DOE method to determine the appropriate culture parameters and obtain better results.

Related Products

Cat.No.	Product
A11002	QuaCell® CHO CD02 Medium
A11004	QuaCell® CHO CD04 Medium
A12004	QuaCell® CHO CD04 Medium
A11902	QuaCell® CHO Feed02 Supplement
A12902	QuaCell® CHO Feed02 Supplement

Explanation of Symbols and Warnings

STERILE A		
Sterilized using aseptic processing techniques	Use By:	Store Temperature
LOT		
Batch code	Dry preservation	Keep away from light
RUO	GMP	
Research use only	GMP Manufacturing	Sticky notes