Product Information

CHO Pro (AC) Medium

Serum-Free, Chemically Defined Medium, w/o L-Glutamine

CHO Pro (AC) cell culture medium has been formulated to provide high yields of recombinant proteins expressed using Chinese hamster ovary (CHO) cell lines such as CHOS, CHO-K1, DG44, or DXB11 cells. CHO PRO (AC) medium is a chemically defined (CD), serum-free (SF), animal origin-free (AOF) medium that contains no protein, hydrolysates, or components of unknown composition. The medium is optimized for high-yield protein production in batch or fed-batch processes. The medium is formulated without hypoxanthine and thymidine for use in dihydrofolate reductase (DHFR)-amplified systems; without L-glutamine for use in glutamine synthetase systems; and without phenol red to minimize estrogen-like effects of phenol red.

Specification Table

Classification	Serum	Animal Origin	Protein	Endotoxin	Antibiotics	Phenol Red	Glutamine
Chemically Defined	Free	Free	Free	< 1 EU/mL	No	No	No

Storage and Shelf-life

CHO Pro (AC) medium is highly hygroscopic powder and should be stored in dry at 2°C to 8°C protected from light and moisture. The entire contents of each package should be used immediately after opening. Please refer to the label for the expiry date.

Culture conditions

Media: CHO Pro (AC)
Cell line: CHO cells
Culture type: Suspension
Temperature range: 36°C to 38°C
Incubator atmosphere: Humidified atmosphere of 5-8% CO2 in air**.**
Culture vessels: Shake flasks, spinner bottles, and bioreactor.

Reconstitute CHO Pro (AC) medium

1. Take 90 % of the final volume of cell culture grade water at an ambient temperature into an appropriately sized mixing vessel.
2. Add the dry powder medium (25.00 g/L) slowly to the water and mix gently for 30-45 minutes.
3. Add 2.1 g of sodium bicarbonate per liter of the final volume of the medium and stir until dissolved (about 20 minutes).
4. Add cell culture grade water to achieve the appropriate final volume and mix well.
5. Measure pH and Osmolality
6. Sterilize the medium immediately by using 0.22 µm filter. Aliquot the sterile solution under aseptic conditions to avoid contamination, and store the medium at 2–8 °C, protected from light for 6 months

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Complete medium preparation for use

1. Supplement CHO Pro (AC) medium with L-glutamine at 4-8 mM final concentration prior to us.
2. CHO Pro (AC) medium contains no hypoxanthine and thymidine. Please supply to the medium if necessary for your process.
3. Glucose supplementation may be required for terminal batch cultures and should be determined empirically.
4. Anti-clumping agent can be added to reduce cell aggregation. Concentration of anti-clumping agent can be determined experimentally for individual cultures or can be supplemented based on suppliers recommendation.

Recover frozen cells

1. Rapidly thaw (about 1 minute) frozen cells

in a 37°C water bath.
2. Transfer the contents of the cryovial into a 125-mL shake flask containing 30 mL complete CHO Pro (AC) Medium.
3. Incubate at 37°C in a humidified atmosphere of 5-8% CO2 in air with shaking speed of 115–135 rpm.
4. Maintain a cell density of 0.5 × 10^6–1.5 × 10^6 viable cells/mL for the first two passages following recovery; thereafter, return to your normal maintenance schedule.

Subculture cells

1. Determine viable cell density using an automated cell counter or manual methods. Ensure that the cell density is ≥1 × 10^6 viable cells/mL, viability is ≥90%, and cells are in the mid-logarithmic phase prior to subculturing.
2. Calculate the volume of cell culture and medium necessary to seed a flask at 2 × 10^5–3 × 10^5 viable cells/mL in a total volume of 30 mL fresh CHO PRO (AC) medium per 125-mL shake flask.
   NOTE: If cell density does not reach 1 × 10^6 viable cells/mL within 5 days of recovery, centrifuge cells at 100 × g for 5 minutes and resuspend the cell pellet in 20–30 mL of fresh complete CHO Pro (AC) medium.
3. Incubate at 37°C in a humidified atmosphere of 5-8% CO2 in air with shaking speed of 115–135 rpm.
4. Subculture cells by seeding at density of 2-3 × 10^5 viable cells/mL every 3–4 days with fresh CHO PRO (AC) medium.

Adaptation of CHO cells to CHO Pro (AC) Medium

We recommend adapting CHO cells to CHO Pro (AC) medium using sequential adaptation. However, some CHO cell lines will adapt directly from other medium, especially those which are being maintained in other serum-free medium. It is critical that cell viability be ≥90% and the growth is in mid-logarithmic phase prior to initiating adaptation procedures.

Sequential adaptation

1. Expand the culture grown in conventional 5–10% serum-supplemented medium or other serum-free medium until cells are in logarithmic phase having viability greater than 90%.
2. Dilute cells by seeding at 4 × 10^5– 5 × 10^5 viable cells/mL in 1:3 (25:75) ratio of complete CHO PRO (AC) medium to the original medium.
3. Subculture cells at 4 × 10^5– 5 × 10^5 viable cells/mL in the same medium when viable cell density reaches ≥1 × 10^6 cells/mL. Once consistent cell growth with high viability (>90%) is achieved, subculture cells into 1:1 (50:50) ratio of complete CHO PRO (AC) medium to the original medium.
4. Repeat step 3 by stepwise reducing the original medium [3:1 (75:25) and 9:1 (90:10)] followed by 100% CHO PRO (AC) medium. Multiple passages at each step may be needed.
5. After several passages in 100% CHO PRO (AC) medium, the viable cell count should reach at least 2 × 10^6 cells/mL with ≥85% viability within 3–4 days of seeding culture. At this stage, the culture is considered to be adapted to CHO Pro (AC) medium.
   NOTE
   - Make a frozen stock of the cells in the original medium prior to adaptation.
   - Keep a culture going of the cells in each prior condition when starting the next level of adaptation as a fall-back if the cells do not survive in the next passage.
   - Make 2-3 vials during each step of sequential adaptation as a back-up.

Direct adaptation

1. For direct adaptation of CHO cells grown in other serum-free medium into CHO Pro (AC) medium, dilute cells into 100% CHO Pro (AC) medium by seeding at 3 × 10^5–4 × 10^5 viable cells/mL when subculturing.
2. Continue to subculture cells at 3 × 10^5–4 × 10^5 viable cells/mL (every 3–4 days) until consistent growth is achieved. Once cell

growth has been demonstrated, the seeding density may be reduced to 2 × 10^5–3 × 10^5 viable cells/mL during the final stages of adaptation.

3. After several passages in CHO Pro (AC) medium, the viable cell count should reach at least 2 × 10^6 cells/mL with ≥85% viability within 3–4 days of seeding culture. At this stage, the culture is considered to be adapted to CHO PRO (AC) medium
   NOTE: If suboptimal performance is achieved using the direct adaptation method, use the sequential adaptation method.

Cryopreservation

1. Prepare the desired quantity of cells, harvesting in mid-log phase of growth having viability >90%.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of >1 × 10^7 cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% CHO Pro (AC) medium + 7.5% DMSO and store at 4°C until use.
   NOTE: Prepare cryopreservation medium on the day of use.
4. Harvest cells by centrifugation at 100 × g for 5–10 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications.
6. Achieve cryopreservation in an automated controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen (vapor phase) storage at –200°C to –125°C.
   NOTE: Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen (see "Recover frozen cells" section).

Precautions and Disclaimer

This product is for research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices. We provide information to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations should be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information does not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

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Help Needed?

For any further questions regarding this product, please contact us at info@sarvoshadhi.com.