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# CD CHO Medium

## Description

CD CHO Medium has been developed for the growth of Chinese Hamster Ovary (CHO) cells and expression of recombinant proteins in suspension culture. CD CHO is an animal origin-free (AOF), chemically defined medium that contains no proteins, hydrolysates, or components of undefined composition. CD CHO is formulated without hypoxanthine and thymidine for use in dihydrofolate reductase (DHFR) amplified systems, and without phenol red to minimize estrogen-like effects of phenol red.

| Product | Catalog no. | Amount | Storage | Shelf life* |
|---|---|---|---|---|
| CD CHO Medium (1X), liquid | 10743-011<br>10743-029 | 500 mL<br>1000 mL | 2°C to 8°C; Protect from light | 18 months |
| | 10743-001<br>10743-002 | 10 L (Bag)<br>20 L (Bag) | 2°C to 8°C; Protect from light | 12 months |
| CD CHO AGT™** | 12490-017<br>12490-025<br>12490-001<br>12490-003 | 1 L<br>1 × 10 L<br>1 × 100 L<br>10 kg | 2°C to 8°C; Store dark and dry | 24 months |

* Shelf Life duration is determined from Date of Manufacture. ** AGT = Advanced Granulation Technology.

## Product use

Caution: For manufacturing, processing, or repacking.

## Safety information

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

## Prepare media

CD CHO Medium and CD CHO AGT™ medium require supplementation with L-glutamine or GlutaMAX™-I prior to use.

1. Aseptically add L-glutamine or GlutaMAX™-I 8 mM final concentration (40 mL/L) to the medium before use.
2. If L-glutamine is not required, add 40 mL of sterile distilled water and adjust the osmolality to 320 mOsm using a sterile solution of NaCl.

   **Note:** Omit step 2, if using CD CHO AGT™.
3. CD CHO Medium is made without hypoxanthine and thymidine for use in dihydrofolate reductase (DHFR) amplified systems. For other applications, add 10 mL/L of HT Supplement prior to use.
4. Add 1 mL/L of Anti-Clumping Agent to media if cell clumping occurs. After any medium changes, passage cells for a minimum of 3X before use in other applications.

   **Note:** Consider using lower levels of L-glutamine if you are using a fed batch or perfusion protocol or if the cell line in use is sensitive to ammonia.

   **Note:** Addition of a surfactant (e.g., Pluronic® F-68) is not required.

## Reconstitute CD CHO AGT™ Medium

1. Weigh out 24.3 g (equivalent to the entire contents of a 1-L package) of CD CHO AGT™ medium.
2. Add to 900 mL room temperature deionized or distilled water. Rinse inside of package to remove all traces of powder. Mix gently for 30 minutes or until medium dissolves completely.
3. Add deionized or distilled water to final volume of 1 L.
4. Filter sterilize by 0.2 µm pore size membrane filtration.

   **Note:** Use low protein binding, low extractables filter.
5. Supplement as described in **Prepare Media** at time of use.

   **Note:** CD CHO AGT™ medium contains sodium bicarbonate. **Do not** add additional sodium bicarbonate. CD CHO AGT™ medium is auto pH and osmolality adjusted, no further adjustment is required. For final lot pH and osmolality specifications please refer to Certificate of Analysis specification.

## Culture conditions

**Media:** CD CHO Medium

**Cell line:** Chinese Hamster Ovary (CHO)

**Culture type:** Suspension

**Culture vessels:** shake flasks, spinner bottles, or bioreactor. Procedures described are intended for use with 125-mL Erlenmeyer shake flasks.

**Temperature range:** 36°C to 38°C

**Incubator atmosphere:** Humidified atmosphere of 8–10% $CO_2$ in air. Ensure proper gas exchange and minimize exposure of cultures to light.

## Recovery

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a 125-mL shake flask containing 28.5 mL of pre-warmed complete CD CHO Medium.
3. Incubate at 37°C in a humidified atmosphere of 8–10% $CO_2$ in air on an orbital shaker platform rotating at 125–135 rpm.
4. Subculture cells in mid-logarithmic phase 3–5 days post-thaw at a seeding density of $3 \times 10^5$ viable cells/mL. Passage cells a minimum of 3X before use in other applications.

   **Note:** Do not centrifuge CHO cells to remove DMSO as they are extremely fragile upon recovery from cryopreservation.

## Subculture suspension cultures

1. Determine viable cell density using a Countess® Automated Cell Counter.
2. Seed cells at $2 \times 10^5$–$3 \times 10^5$ viable cells/mL in sterile culture vessels containing pre-warmed complete CD CHO Medium. (30 mL per 125-mL shake flask).
3. Incubate at 37°C in a humidified atmosphere of 8–10% $CO_2$ in air on an orbital shaker platform rotating at 125–135 rpm. Loosen flask cap to allow for gas exchange.
4. Subculture cells when viable cell density reaches ≥$1 \times 10^6$ viable cells/mL into clean, sterile flask(s) with fresh pre-warmed complete CD CHO Medium.

   **Note:** To reduce accumulation of cell debris and metabolic waste by-products in suspension cultures, gently centrifuge the cell suspension at $100 \times g$ for 5–10 minutes and resuspend pellet in fresh complete CD CHO Medium once every 2–3 weeks.

   **Note:** It is recommended to thaw a fresh low-passage vial of cells every 3 months or 30 passages.

## Adapt CHO Cells to CD CHO Medium

It is critical that cells be in mid-logarithmic phase growth and exceed 90% viability prior to initiating adaptation procedures from conventional serum-supplemented or serum-free medium.

### Direct adaptation

Transfer suspension cultures into CD CHO Medium as follows:

1. Centrifuge the cell suspension at 100 × *g* for 5–10 minutes. Aspirate and discard the supernatant.
2. Resuspend the cell pellet in pre-warmed complete CD CHO Medium at a viable cell density of $3 \times 10^5$–$5 \times 10^5$ cells/mL and transfer to appropriate culture vessels.
3. Return to incubator and monitor cell growth.

   **Note:** If suboptimal cell growth is observed using the direct adaptation method, use the sequential adaptation method.

### Sequential adaptation

1. Follow the procedures for **Subculture Suspension Cultures** with the following modifications.
2. During the adaptation procedure use a seeding density of $4 \times 10^5$–$5 \times 10^5$ viable cells/mL.
3. Subculture cells into stepwise increasing ratios of complete CD CHO Medium to original medium with each subsequent passage (25:75, 50:50, 75:25, 90:10 followed by 100% CD CHO Medium). Multiple passages at each step may be needed.
4. After several passages in 100% CD CHO Medium, the viable cell count should exceed $1 \times 10^6$–$2 \times 10^6$ cells/mL with a viability ≥85% within 4–6 days of culture. At this stage the culture is considered to be adapted to CD CHO Medium. The seeding density may be reduced to $2 \times 10^5$–$3 \times 10^5$ viable cells/mL during the final stages of adaptation.

## Cryopreservation

Prepare the desired quantity of cells, harvesting in mid-log phase of growth with viability >90%.

1. Obtain Synth-a-Freeze® cryopreservation medium and store at 2°C to 8°C until use.
2. Determine the viable cell density and calculate the required volume of Synth-a-Freeze® cryopreservation medium. Typical cell densities for cryopreservation with Synth-a-Freeze® medium are $0.5 \times 10^7$–$1 \times 10^7$ viable cells/mL.
3. Harvest cells by centrifugation at 100 × *g* for 5–10 minutes. Resuspend cell pellet in the pre-determined volume of 2°C to 8°C of Synth-a-Freeze® medium.
4. Immediately dispense aliquots of this suspension into cryovials according to the manufacturer's specifications
5. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
6. Transfer frozen cells to liquid nitrogen; (vapor phase) storage at –200°C to –125°C is recommended.

   **Note:** Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen. See **Recovery**.

## Related products

| Product | Catalog No. |
|---|---|
| L–Glutamine, 200mM (100X), liquid | 25030 |
| GlutaMAX™-I, 200mM (100X), liquid | 35050 |
| HT Supplement, (100X), liquid | 11067 |
| Anti-Clumping Agent | 0010057 |
| CD DG44 Medium, (1X) liquid | 12610 |
| CHO CD EfficientFeed™ Kit | A10241 |
| CHO CD EfficientFeed™ A AGT™ Nutrient Supplement | A14420 |
| CHO CD EfficientFeed™ B AGT™ Nutrient Supplement | A12456 |
| CD EfficientFeed™ C AGT™ Nutrient Supplement | A13275 |
| Water, Distilled | 15230 |
| CHO-S™ Cells (cGMP banked) and Media Kit | A11557 |
| Countess® Automated Cell Counter | C10227 |

## Explanation of symbols and warnings

The symbols present on the product label are explained below:

| | | | | |
|---|---|---|---|---|
|  |  |  |  |  |
| Catalog number | Manufacturer | Use by | Keep away from light | Batch code |

| | | | |
|---|---|---|---|
|  |  |  |  |
| Caution, consult accompanying documents | Consult instructions for use | Temperature limitation | Sterilized using aseptic processing techniques |

## Limited product warranty

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## Limited Use Label License: Internal Research and Bioproduction Use

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product (a) to perform internal research for the sole benefit of the purchaser; and (b) to culture cells for the purpose of producing a product wherein the product will be used for any or all of the following: (i) internal research use by the purchaser; (ii) resale for internal research use by third parties; (iii) performance of research conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties; (iv) resale for use as a human therapeutic agent or diagnostics product or component by third parties; (v) performance of manufacturing services conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties.

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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit **www.lifetechnologies.com/support**.
For further assistance, email **techsupport@lifetech.com**.



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