# CHO-S™ Cells (cGMP Banked) and Media Kit

**Catalog Number** A11557-01

**Pub. No.** MAN0007378 **Rev.** 3.0

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

## Product description

Gibco™ CHO-S™ Cells (cGMP Banked) and Media Kit have been developed for the growth of Chinese Hamster Ovary (CHO) cells and expression of recombinant proteins in suspension culture. CHO-S™ cells have been adapted to CD CHO Medium for serum–free suspension growth, and subsequently banked and tested to meet cGMP quality standards. CD CHO Medium is an animal origin-free (AOF), chemically defined medium that contains no proteins, hydrolysates, or components of unknown composition.CD CHO Medium is formulated without L-glutamine for greater stability, and without phenol red to minimize potential for estrogen-like effects. CD CHO Medium is made without hypoxanthine and thymidine for use in dihydrofolate reductase (DHFR) amplified systems.

## Contents and storage

**Table 1** CHO-S™ Cells (cGMP Banked) and Media Kit, Cat. No. A11557-01

| Contents | Cat. No. | Amount | Storage | Shelf life[1] |
|---|---|---|---|---|
| CD CHO Medium | 10743-029 | 1000 mL | 2°C to 8°C. Protect from light. | 18 months |
| CHO-S™ Cells (cGMP Banked) | A11364-01 | 1 vial[2] | –200°C to –125°C. Liquid Nitrogen. | — |
| L-Glutamine, 200 mM | A2916801 | 100 mL | –20°C to –5°C. Protect from light. | 24 months |

[1] Shelf-Life duration is determined from Date of Manufacture.
[2] 1 vial contains ≥ $1 \times 10^7$ cells/vial

## Important information

- CHO-S™ cells have been produced, banked, and tested to meet current Good Manufacturing Practice regulations 21 CFR Parts 210, 211, 600, and 610.
- CHO-S™ Cells: Stable when maintained at –200°C to –125°C.

## Prepare medium

CD CHO Medium requires supplementation with L-glutamine prior to use.

1. Aseptically add L-glutamine to 8 mM final concentration (40 mL/L), to the medium before use.
2. If cell clumping occurs, add 1 mL/L of Anti-Clumping Agent to medium. After any thaw or changes in media composition, subculture cells for a minimum of 3 passages before use in other applications.

**Note:** Consider reducing L-glutamine concentration for fed batch or perfusion protocols, or to reduce ammonia levels.

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

## Culture conditions

**Medium**: Complete CD CHO Medium

**Cell line**: CHO-S™ Cells (cGMP Banked)

**Culture type**: Suspension

**Culture vessels**: Shake flask or spinner bottle

**Temperature range**: 36°C to 38°C

**Incubator atmosphere**: Humidified atmosphere of 5–10% $CO_2$ in air. Ensure proper gas exchange and minimize exposure of cultures to light.

## Recovery

1. Rapidly thaw (<2 minutes) frozen vial of cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a 125-mL shake flask containing 29 mL of prewarmed complete CD CHO Medium.

   If thawed properly, cell density should be ≥$3 \times 10^5$ viable cells/mL, and viability should be ≥90%.

3. Incubate at 37°C in a humidified atmosphere of 5–10% $CO_2$ in air on an orbital shaker platform rotating at 125–135 rpm. Loosen flask caps (or use vented caps) to allow for gas exchange.

4. Subculture cells, 2–3 days post-thaw, when viable cell density reaches 1 × $10^6$ cells/mL in mid-logarithmic phase of growth. Seed cultures at a density of 3 × $10^5$ viable cells/mL. Subculture cells a minimum of 3 passages before use in other applications.

   **Note:** Do not centrifuge CHO-S™ cells as they are extremely fragile upon recovery from cryopreservation.

## Scaling up CHO-S™ Cells in CD CHO Medium

CHO-S™ cultures can be scaled up in spinner bottles or stirred tank bioreactors using the following guidelines.

- Determine the optimum spinner or impeller speed for your bioreactor depending on culture requirements.
- Seeding density: We recommend an optimized seeding density of 1–2 × $10^5$ viable cells/mL.

  **Note:** If the split ratio of cells to fresh media is <1:2, we recommend to spin down the cell suspension at 100 × g for 5–10 minutes, and resuspending the cell pellet in fresh complete CD CHO Medium prior to inoculating the spinner or bioreactor culture.

## Cryopreservation

Prepare the desired quantity of cells in a tissue culture flask, harvesting in mid-log phase of growth when viable cell density reaches >1 × $10^6$ cells/mL with viability >90%.

1. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final viable cell density of ≥1 × $10^7$ cells/mL.

2. Prepare the required volume of cryopreservation medium (90% fresh complete, and 10% DMSO) and store at 4°C until use.

   **IMPORTANT!** Prepare cryopreservation medium on the day of use.

3. Harvest cells by centrifugation at 100 × g for 5–10 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.

4. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1.5 mL in a 2-mL cryovial).

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.

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

## Related products

| Product | Catalog No. |
|---|---|
| Anti-Clumping Agent | 0010057AE |
| FreeStyle™ MAX Reagent | 16447 |
| FreeStyle™ MAXCHO Expression System | K9000-20 |
| EfficientFeed™ A+ AGT™ Supplement | A25023 |
| EfficientFeed™ B+ AGT™ Supplement | A25030 |
| EfficientFeed™C+ AGT™ Supplement | A25031 |
| CD CHO AGT™ Medium | 12490 |
| CD CHO Medium (1X), Liquid | 10743 |
| Water, Distilled | 15230 |
| Freedom™ CHO-S™ Kit | A13696 |
| Countess™ Automated Cell Counter | C10227 |
| Trypan Blue Stain | 15250 |

## Explanation of symbols

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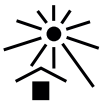
|---|---|---|---|---|
|  |  |  |  |  |
| Temperature Limitation | Manufacturer | Batch code | Use By | Catalog Number |
|  |  |  |  | |
| Caution, consult accompanying documents | Consult instructions for use | Keep away from light | Sterilized using aseptic processing techniques | |

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