CD CHO Medium

Catalog Numbers 10743, 12490

Pub. No. MAN0007311 **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™ 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 Medium is an animal origin-free (AOF), chemically defined medium that contains no proteins, hydrolysates, or components of undefined composition. CD CHO Medium 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.

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf life[1]
CD CHO Medium	10743011 10743029	500 mL 1000 mL	2°C to 8°C; Protect from light	18 months
	10743001 10743002	10 L (Bag) 20 L (Bag)	2°C to 8°C; Protect from light	12 months
CD CHO AGT™ Medium[2]	12490017 12490025 12490001 12490003	1 L 1 × 10 L 1 × 100 L 10 kg	2°C to 8°C; Store dark and dry	24 months

[1] Shelf Life duration is determined from Date of Manufacture.
[2] AGT = Advanced Granulation Technology.

Prepare media

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

1. Aseptically add L-glutamine or GlutaMAX™ Supplement 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™ Medium.
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.
3. Mix gently for 30 minutes or until medium dissolves completely.

4. Add deionized or distilled water to final volume of 1 L.
5. Filter sterilize by 0.2 µm pore size membrane filtration.

   Use low protein binding, low extractables filter.
6. Supplement as described in "Prepare media" at time of use.

   Note: CD CHO AGT™ Medium contains sodium bicarbonate.

IMPORTANT! 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.

Recover cells

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 3×10^5 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™ II Automated Cell Counter.
2. Seed cells at 2×10^5–3×10^5 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.

   Loosen flask cap to allow for gas exchange.
4. Subculture cells when viable cell density reaches ≥1×10^6 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 × *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.
2. Aspirate and discard the supernatant.
3. Resuspend the cell pellet in pre-warmed complete CD CHO Medium at a viable cell density of 3×10^5–5×10^5 cells/mL and transfer to appropriate culture vessels.
4. 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 4×10^5–5×10^5 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 1×10^6–2×10^6 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 2×10^5–3×10^5 viable cells/mL during the final stages of adaptation.

Cryopreserve cells

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

1. Store Synth-a-Freeze™ Cryopreservation Medium 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™ Cryopreservation Medium are 0.5×10^7–1×10^7 viable cells/mL.
3. Harvest cells by centrifugation at 100 × *g* for 5–10 minutes.
4. Resuspend cell pellet in the pre-determined volume of 2°C to 8°C of Synth-a-Freeze™ Cryopreservation Medium.
5. Immediately dispense aliquots of this suspension into cryovials according to the manufacturer's specifications.
6. Achieve cryopreservation in an automated or manual 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 is recommended.

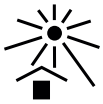
   Note: Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen. See "Recover cells".

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
L-Glutamine (200 mM)	25030
GlutaMAX™ Supplement (200 mM)	35050
HT Supplement (100X)	11067
Anti-Clumping Agent	0010057
CD DG44 Medium	12610
CHO CD EfficientFeed™ Kit	A10241
CHO CD EfficientFeed™ A AGT™ Nutrient Supplement	A1442001
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™ II Automated Cell Counter	AMQAX1000
Synth-a-Freeze™ Cryopreservation Medium	A1254201

Explanation of symbols

Symbol	Description	Symbol	Description	Symbol	Description
	Manufacturer		Catalog number		Batch code
	Use by		Temperature limitation		Keep away from light
	Sterilized using aseptic processing techniques		Consult instructions for use		Caution, consult accompanying documents

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Manufacturer: Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072

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