ExpiCHO™ Stable Production AGT™ Medium

Catalog Numbers A3711101, A3711102, A3711103

Pub. No. MAN0017637 **Rev.** 2.0

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™ ExpiCHO™ Stable Production AGT™ Medium is a chemically-defined, protein-free, animal origin component-free medium developed specifically to support high titer expression of stable ExpiCHO-S™ clones in suspension. The medium is designed to provide a seamless scale-up solution for customers using the ExpiCHO™ Expression System for transient production looking to transition to the development of stable ExpiCHO™ clones. The medium is formulated without hypoxanthine and thymidine for use in dihydrofolate reductase (DHFR)-amplified systems, without L-glutamine or GlutaMAX™ I Supplement for use in glutamine synthetase systems, and without phenol red in order to minimize the estrogen-like effects of phenol red.

ExpiCHO™ Stable Production AGT™ Medium allows for direct transition from transient expression to the large scale production of stable clones with no adaptation. You can start process development faster, and streamline or simplify transfer to manufacturing scale.

Note: ExpiCHO™ Stable Production AGT™ Medium is not compatible for use as a medium during the transfection stage.

Contents and storage

Contents	Cat. No.	Amount	Storage
ExpiCHO™ Stable Production AGT™ Medium	A3711101	10 L	2–8°C; Protect from light.
	A3711102	100 L	
	A3711103	450 L	

Procedural guidelines

- Store the frozen cells in liquid nitrogen until ready to use. Do not store the cells at –80°C.
- ExpiCHO™ Stable Production AGT™ Medium is sensitive to light. For optimal results, use and store media protected from light.

Culture conditions

Medium: ExpiCHO™ Stable Production AGT™ Medium

Culture type: Suspension

Temperature range: 37°C ±5°C

Shaker speed: For shakers with a 19-mm throw, set the shake speed to 125 ±5 rpm. For shakers with a 25-mm throw, set the shake speed to 120 ±5 rpm.

Incubator atmosphere: Humidified atmosphere of 8% CO_2. Ensure that proper gas exchange is achieved in culture vessels.

Guidelines to prepare medium

ExpiCHO™ Stable Production AGT™ Medium requires aseptic supplementation with GlutaMAX™ I Supplement.

- Add GlutaMAX™ I Supplement at 2–8 mM final concentration to the medium before use.
- Glucose supplementation may be required for terminal batch cultures and should be determined empirically.

Reconstitute ExpiCHO™ Stable Production AGT™ Medium

1. Measure 90% of the final volume deionized or distilled water at room temperature (15°C to 30°C).
2. Add ExpiCHO™ Stable Production AGT™ Medium at 23.9 g/L to water.
3. Mix for a minimum of 30 minutes.
4. Dilute to final production volume with ambient deionized or distilled water, using a calibrated vessel.
5. Mix for an additional 10 minutes.

6. Measure the pH and check and record osmolality. pH should be in the range of 6.8 - 7.4. Osmolality should be in the range of 285-305.

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7. Sterilize immediately by membrane filtration (positive pressure recommended).

   Note: Once the product is filtered, use immediately or store at 2 to 8°C for up to 6 months. Protect from light.

Guidelines for ExpiCHO-S™ cell culture

- ExpiCHO-S™ is a robust cell line adapted to high density growth conditions with a doubling time of approximately 17 hours.
- The cells have a broad log-phase growth window spanning approximately 4×10^6–15×10^6 cells/mL with a maximum density of ≥20×10^6 cells/mL in shake flask cultures.
- For general maintenance of cells, passage ExpiCHO-S™ cells when they reach a density of approximately 4×10^6–6×10^6 viable cells/mL (i.e. early log-phase growth), typically every 3–4 days.
- Cells that are subcultured at densities outside of this early log-phase growth window may show longer doubling times and lower titers over time. If necessary, modify the initial seeding density to attain the target cell density of 4×10^6–6×10^6 viable cells/mL at the time of subculturing.
- Use a hemocytometer with the trypan blue exclusion method or an automated cell counter to determine cell viability. Log phase cultures should be >95% viable.
- When thawing or subculturing cells, transfer cells into pre-warmed medium.

Thaw ExpiCHO-S™ cells

1. Remove the vial of cells from liquid nitrogen and swirl in a 37°C water bath for 1 to 2 minutes to thaw the cells rapidly until only a small amount of ice remains.

   IMPORTANT! Do not submerge the vial in the water.

2. Just before the cells are completely thawed, decontaminate the vial by wiping it with 70% ethanol before opening it in a laminar flow hood.

3. Use a 2-mL or 5-mL pipette, transfer the entire contents of the cryovial into a 125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask containing 30 mL of pre-warmed ExpiCHO™ Stable Production AGT™ Medium.

4. Incubate the cells in a 37°C incubator with humidified atmosphere of 8% CO_2 on an orbital shaker platform.

5. Three days post-thaw, determine viable cell density and percent viability.

   Cell viability should be >90% by three days post-thaw.

6. Continue to monitor cell density and viability and subculture the cells once the culture has reached 4×10^6–6×10^6 viable cells/mL (typically 3–4 days post-thaw).

Subculture ExpiCHO-S™ cells

Subculture ExpiCHO-S™ cells when they attain a minimum density of 4×10^6–6×10^6 viable cells/mL. Cells should exhibit only minimal clumping during routine cell culture maintenance.

1. Using the viable cell density, calculate the volume of cell suspension required to seed a new shake flask according to the recommended seeding densities in Table 1 and the recommended culture volumes in Table 2.

2. Transfer the calculated volume of cells to fresh, pre-warmed ExpiCHO™ Stable Production AGT™ Medium in a shake flask.

3. Incubate flasks in a 37°C incubator with a humidified atmosphere of 8% CO_2 on an orbital shaker platform until cultures reach a density of 4×10^6–6×10^6 viable cells/mL.

   Note: If necessary, modify the initial seeding density to attain the target cell density of 4×10^6–6×10^6 viable cells/mL at the time of subculturing.

4. Repeat Steps 1–3 to maintain or expand the cells for transfection.

Table 1 Recommended seeding densities for routine cell culture

Subculture timing	Seeding density
Cells ready 3 days post-passage	0.2×10^6–0.3×10^6 viable cells/mL
Cells ready 4 days post-passage	0.1×10^6–0.2×10^6 viable cells/mL

Table 2 Recommended culture volumes for various flask sizes

Flask size	Recommended culture volume
125-mL	30–35 mL
250-mL	60–70 mL
500-mL	120–140 mL
1-L	240–260 mL

Cryopreserve cells

ExpiCHO-S™ cells can be frozen directly in ExpiCHO™ Stable Production AGT™ Medium.

1. Freeze ExpiCHO-S™ cells at a final density of 1×10^7 viable cells/mL in 1 mL total volume of 90% fresh ExpiCHO™ Stable Production AGT™ Medium and 10% DMSO.
2. Allow cells to attain a viable cell density of 4×10^6–6×10^6 cells/mL and >95% viability before harvest.
3. Centrifuge the cells at 300 × *g* for 5 minutes to pellet, discard the spent medium, and replace it with ice cold ExpiCHO™ Stable Production AGT™ Medium with 10% DMSO.
4. Gently resuspend the cell pellet by pipetting.
5. Dilute the cells to a final density of 1×10^7 viable cells/mL and aliquot 1 mL per cryovial.
6. Freeze the cells in an automated or manual controlled-rate freezing apparatus following standard procedures.

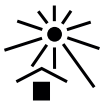
   For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.
7. Transfer frozen vials to liquid nitrogen for long-term storage.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
ExpiCHO™ Expression System	A29133
ExpiFectamine™ CHO Transfection Kit	A29129
ExpiCHO-S™ Cells (1×10^7 cells/mL)	A29127
ExpiCHO™ Expression Medium	A29100
Trypan Blue Stain	15250
GlutaMAX™ I Supplement	35050061
EfficientFeed™ C+ AGT™ Supplement	A25031

Explanation of symbols

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

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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