Expi293™ Expression Medium

Catalog Numbers A1435101, A1435102, A1435103, A1435104

Pub. No. MAN0006285 **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**.

Production description

Gibco™ Expi293™ Expression Medium is an optimized, chemically defined formulation designed to support the high-density culture and transfection of 293 cells (e.g., Expi293F™ cells) in suspension. This chemically defined medium does not contain any protein, undefined lysates or components of animal origin. Expi293™ Expression Medium is a complete, ready-to-use medium formulated with GlutaMAX™ Supplement, and it requires no supplementation. The medium is not recommended for adherent 293 cell culture.

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf Life[1]
Expi293™ Expression Medium	A1435101	1000 mL	2–8°C; Protect from light.	12 months
	A1435102	6 × 1000 mL		
	A1435103	10 L		
	A1435104	20 L		

[1] Shelf Life duration is determined from Date of Manufacture.

Culture conditions

Media: Expi293™ Expression Medium

Cell line: Expi293™ Cells; other 293 cell lines (e.g., FreeStyle™ 293-F Cells) may also be used with adaptation

Culture type: Suspension

Culture vessel: It is recommended to use PETG or polycarbonate, non-baffled, vented Erlenmeyer flasks; however, baffled Erlenmeyer flasks may also be used. Cultures may also be sealed up in spinner flasks or bioreactors.

Temperature range: 37°C ±0.5°C

Shaker speed: For shakers with a 19-mm shaking diameter, set the shake speed to 125 ±5 rpm. For shakers with a 25-mm shaking diameter, set shake speed at 120 ±5 rpm. For shakers with a 50-mm shaking diameter, set the shake speed to 95 ±5 rpm.

Incubator atmosphere: ≥80% humidified, 8% CO_2 atmosphere. Ensure proper gas exchange and minimize exposure of cultures to light.

Procedural guidelines

- Expi293™ Expression Medium contains GlutaMAX™ Supplement and it does not require further supplementation with L-glutamine or GlutaMAX™ Supplement.
- Expi293™ Expression Medium is sensitive to light; use and store the medium protected from light.
- Antibiotics are not recommended; however, 5 mL/L of Antibiotic-Antimycotic (Cat. No. 15240) containing penicillin, streptomycin, and amphotericin B may be used when required.
- Passage Expi293F™ cells directly into Expi293™ Expression Medium.
- When maintaining Expi293F™ cells, it is recommended to use a 125-mL or 250-mL PETG or polycarbonate, disposable, sterile Erlenmeyer flask containing 20–32% total working volume of cell suspension. When using larger flasks, the total working volume should be between 25–33%.

Thaw

1. Rapidly thaw (1–2 minutes) a frozen vial of Expi293F™ cells in a 37°C water bath.

   Note: We recommend thawing the vial in a 125-mL shaker flask.

2. Decontaminate the vial with 70% isopropyl alcohol or ethanol, and transfer the entire contents of the cryovial into a 125-mL shaker flask containing 30 mL of pre-warmed Expi293™ Expression Medium.

3. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker platform. See "Culture conditions" for shake speed recommendations.

4. Allow cells to culture for 3–4 days post-thaw, then determine the viable cell density and percent viability.

   Note: The viability of the cells may drop slightly 24 hours post-thaw but should remain above 70% and reach over 90% within 4–7 days post-thaw.

5. Subculture the cells when the cell density reaches ≥1 × 10^6 cells/mL and the cells are ≥90% viable (usually 4–7 days post-thaw).

Subculture

1. Determine viable cell density using a hemocytometer or an automated cell counter.

2. Perform the first passage when the cell density reaches ≥ 1 × 10^6 viable cells/mL (typically 4–7 days post-thaw) by seeding shaker flasks at 0.3 × 10^6–0.5 × 10^6 viable cells/mL in fresh, pre-warmed Expi293™ Expression Medium in the desired final volume.

   For subsequent passages, allow the cell density to reach > 3 × 10^6 viable cells/mL (typically 3–4 days) and dilute the cells in fresh, pre-warmed Expi293™ Expression Medium to give a final cell density of 0.3 × 10^6–0.5 × 10^6 viable cells/mL in the desired final volume.

   Note: Do not allow cells to grow above 5 × 10^6 viable cells/mL during maintenance culture.

3. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker platform. See "Culture conditions" for shake speed recommendations.

4. Subculture the Expi293F™ cells a minimum of two additional times to allow them to recover from thawing before using them for transfections or cryopreservation.

Cryopreservation

- Freeze Expi293F™ cells at a final density of 1 × 10^7 viable cells/mL.
- Use a freezing medium composed of 90% fresh Expi293™ Expression Medium and 10% DMSO.
- Freeze 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.
- (*Optional*): Conditioned medium obtained following centrifugation of the cells before freeze down can be added to fresh Expi293™ Expression Medium in the following ratios: 45% fresh Expi293™ Expression Medium, 45% conditioned medium, and 10% DMSO to generate a conditioned freeze medium.
- Transfer frozen vials to liquid nitrogen for long-term storage.

Guidelines for scaling up Expi293F™ cell culture

- It is possible to scale up the Expi293F™ cultures in spinner flasks or bioreactors. The appropriate spinner or impeller speed and seeding density should be determined and optimized for each system.
- The optimum spinner speed has been determined to be 100–130 rpm, and impeller speed in Celligen™ stirred tank bioreactors to be 70–100 rpm.
- We recommend seeding the cells at 0.3 × 10^6–0.5 × 10^6 viable cells/mL.
- If the split ratio of cells to fresh media is less than 1:2, centrifuge the cell suspension and resuspend the cell pellet in fresh, pre-warmed Expi293™ Expression Medium prior to inoculating the spinner or bioreactor culture.
- Monitor cell viability and the degree of cell clumping.
- Extensive cell clumping may reduce transfection efficiency.

Adapt FreeStyle™ 293-F cells to Expi293™ Expression Medium

Pre-warm FreeStyle™ 293 Expression Medium and Expi293™ Expression Medium to 37°C prior to use.

1. Thaw FreeStyle™ 293-F cells in a 125-mL PETG or polycarbonate, disposable, sterile, Erlenmeyer shaker flask with a vented cap containing 29 mL FreeStyle™ 293 Expression Medium following the standard procedure.

2. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker.

3. 24 hours after thawing, determine the viable cell count using a hemocytometer with the trypan blue exclusion method or an automated cell counter.

   Note: Generally, viability of FreeStyle™ 293-F cells after thawing is ≥70%. If the viability is less than 60%, thaw a new batch of cells.

4. Subculture the cells by seeding shaker flasks at 0.3 × 10^6 cells/mL in fresh FreeStyle™ 293 Expression Medium, prewarmed to 37°C. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker.

5. When the culture reaches ≥2 × 10^6 cells/mL with ≥90% viability (3–4 days), passage the cells by seeding shaker flasks at 0.6 × 10^6 viable cells/mL in 10 mL Expi293™ Expression Medium and 20 mL FreeStyle™ 293 Expression Medium (i.e., 1/3 new medium and 2/3 old medium).

6. When the culture reaches ≥3 × 10^6 cells/mL with ≥90% viability (3–4 days), passage the cells by seeding shaker flasks at 0.5 × 10^6 viable cells/mL in 20 mL Expi293™ Expression Medium and 10 mL FreeStyle™ 293 Expression Medium (i.e., 2/3 new medium and 1/3 old medium).

7. When the culture reaches ≥3 × 10^6 cells/mL with ≥90% viability (3–4 days), passage the cells by seeding shaker flasks at 0.4 × 10^6 viable cells/mL in 30 mL Expi293™ Expression Medium (i.e., 100% new medium).

8. Subculture the cells 2 more passages at 0.3 × 10^6 viable cells/mL in 30 mL Expi293™ Expression Medium before using them for transfection.

Related products

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

Item	Cat. No.
Expi293F™ Cells (1 × 10^7 cells/vial)	A14527
Expi293F™ Cells 6 × 1 vial (1 × 10^7 cells/vial)	A14528
Expi293F™ Cells (cGMP banked)	100044202
FreeStyle™ 293-F Cells	R79007
ExpiFectamine™ 293 Transfection Kit, for 1 L of culture	A14524
ExpiFectamine™ 293 Transfection Kit, for 10 L of culture	A14525
ExpiFectamine™ 293 Transfection Kit, for 50 L of culture	A14526
Opti-MEM™ I Reduced Serum Medium	31985062
Opti-Plex™ Complexation Buffer	A4096801
Expi293™ MembranePro™ Expression System (10 reactions)	A25869
Expi293™ MembranePro™ Expression System (100 reactions)	A25870
Trypan Blue Solution, 0.4%	15250061

Limited product warranty

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