

Expi293™ Expression Medium

Catalog Numbers A1435101, A1435102, A1435103, A1435104

Pub. No. MAN0006285 Rev. 4.0



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](https://www.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% CO₂ 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% CO₂ 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 $\geq 1 \times 10^6$ 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 $\geq 1 \times 10^6$ 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 $> 3 \times 10^6$ 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% CO₂ 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% CO₂ 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% CO₂ on an orbital shaker.

5. When the culture reaches $\geq 2 \times 10^6$ cells/mL with $\geq 90\%$ 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 $\geq 3 \times 10^6$ cells/mL with $\geq 90\%$ 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 $\geq 3 \times 10^6$ cells/mL with $\geq 90\%$ 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.

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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



Revision history: Pub. No. MAN0006285 4.0

Revision	Date	Description
4.0	12 July 2023	Changed use statement to: For Research Use or Further Manufacturing. Not for diagnostic use or direct administration into humans or animals.
3.0	8 July 2019	Rebrand, update use to RUO; update: Culture conditions, Thaw, Subculture; Cryopreservation; Adapting cells; add Related products
2.0	27 August 2014	Baseline for this revision.

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