

Expi293™ Expression Medium

Description

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™-I reagent, and it requires no supplementation. The medium is not recommended for adherent 293 cell culture.

Product	Catalog No.	Amount	Storage	Shelf Life*
Expi293™ Expression Medium	A14351-01	1000 mL	2°C to 8°C; Protect from light.	12 months
	A14351-02	6 × 1000 mL		
	A14351-03	10L		
	A14351-04	20L		

* Shelf Life duration is determined from Date of Manufacture.

Product Use

CAUTION: For manufacturing, processing, or repacking.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare Media

- Expi293™ Expression Medium contains GlutaMAX™-I reagent and it does not require further supplementation with L-glutamine or GlutaMAX™-I reagent.
- Expi293™ Expression Medium is extremely 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.

Culture Conditions

Media: Expi293™ Expression Medium

Cell Line: Expi293F™ Cells; other 293 cell lines (e.g., FreeStyle™ 293-F Cells) may also be used with adaptation.

Culture Type: Suspension

Culture Vessel: Vent-cap shaker flask or other appropriate vessel for culture of suspension cells; cultures may also be scaled up in spinner flasks or bioreactors.

Temperature Range: 36°C to 38°C

Incubator Atmosphere: Humidified atmosphere of 8% CO₂ in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Recovery

- Rapidly thaw (1–2 minutes) frozen vial of Expi293F™ Cells in a 37°C water bath. **Note:** We recommend thawing the vial in a 125 mL shaker flask.
- Decontaminate the vial with 70% isopropyl alcohol or ethanol, and transfer the entire contents of the cryovial into a 125-mL vent-cap shaker flask containing 29 mL of pre-warmed Expi293™ Expression Medium.
- Immediately post-thaw, monitor viable cell density and viability using a hemocytometer and the trypan blue exclusion method or using an automated cell counter. Cell density should be approximately 0.3 × 10⁶ cells/mL and cell viability should be above 80%.

- Incubate the cells in a 37°C incubator with humidified atmosphere of 8% CO₂ in air on an orbital shaker platform rotating at 125 rpm ± 5%.
- 24 hours post-thaw, monitor viable cell density and viability using a hemocytometer or an automated cell counter. The viability of the cells will drop slightly but should remain above 70% and reach over 90% by day 2–4 post-thaw. Thaw a new vial of Expi293F™ Cells if cell viability is below 70%.
- Subculture the cells when the cell density reaches ≥1 × 10⁶ cells/mL and the cells are ≥90% viable (usually 2–4 days post-thaw).

Subculture

Passage Expi293F™ Cells directly into Expi293™ Expression Medium. When maintaining Expi293F™ Cells, it is recommended to use a 125-mL or 250-mL 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%.

- Determine viable cell density using a hemocytometer or an automated cell counter.
- Perform the first passage when the cell density reaches >1 × 10⁶ viable cells/mL (typically 2–4 days post-thaw) by seeding shaker flasks at 0.3 × 10⁶ viable cells/mL in pre-warmed Expi293™ Expression Medium in the desired final volume.
For subsequent passages, allow the cell density to reach >3 × 10⁶ 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 × 10⁶–0.5 × 10⁶ viable cells/mL in the desired final volume.
- Incubate the cells at 37°C in a humidified atmosphere of 8% CO₂ in air on an orbital shaker platform rotating at 125 rpm ± 5%.
- 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

Expi293F™ Cells can be frozen directly in Expi293™ Expression Medium. When freezing the Expi293F™ cell line, follow the recommendations below:

- 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.
- Transfer frozen vials to liquid nitrogen for long-term storage.

Note: Check the viability and recovery of frozen cells 24 hours after storing cryovials in liquid nitrogen by following the procedure outlined in **Recovery**.

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. At Life Technologies, 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.

Note: 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. Note that extensive cell clumping may reduce transfection efficiency.

Adapting 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 polycarbonate, disposable, sterile, Erlenmeyer shaker flask with a vented cap containing 29 mL of FreeStyle™ 293 Expression Medium following the standard procedure.
2. Incubate the cells in a 37°C incubator with humidified atmosphere of 8% CO₂ in air on an orbital shaker platform rotating at 125 rpm ± 5%.
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, pre-warmed to 37°C. Incubate the cells in a 37°C incubator with humidified atmosphere of 8% CO₂ in air on an orbital shaker platform rotating at 125 rpm ± 5%.
5. When the culture reaches $>2 \times 10^6$ cells/mL with >90% viability (3–4 days), passage the cells by seeding shaker flasks at 0.6×10^6 cells/mL in 10 mL of Expi293™ Expression

Medium and 20 mL of FreeStyle™ 293 Expression Medium (i.e., 1/3 new medium and 2/3 old medium).

6. When the culture reaches $>3 \times 10^6$ cells/mL with >90% viability (3–4 days), passage the cells by seeding shaker flasks at 0.5×10^6 cells/mL in 20 mL of Expi293™ Expression Medium and 10 mL of FreeStyle™ 293 Expression Medium (i.e., 2/3 new medium and 1/3 old medium).
7. When the culture reaches $>3 \times 10^6$ cells/mL with >90% viability (3–4 days), passage the cells by seeding shaker flasks at 0.4×10^6 cells/mL in 30 mL of Expi293™ Expression Medium (i.e., 100% new medium).
8. Subculture the cells 2 more passages at 0.3×10^6 cells/mL in 30 mL of Expi293™ Expression Medium before using them for transfection.

Related Products

Product	Cat. No.
Expi293F™ Cells (1×10^7 cells/vial)	A14527
Expi293F™ Cells, 6 vial “Cell Bank” pack (1×10^7 cells/vial)	A14528
FreeStyle™ 293-F Cells	R790-07
Expi293™ MembranePro™ Expression System, 10 reactions	A25869
Expi293™ MembranePro™ Expression System, 100 reactions	A25870
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 (5 × 10 L kit)	A14526
Opti-MEM® I Reduced Serum Medium (1X), liquid	31985
Trypan Blue Stain	15250

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

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

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.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

For further information on Expi293 including additional applications and protocols, visit www.lifetechnologies.com/exp293. For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support. For further assistance, email techsupport@lifetech.com

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