









Expi293F™ Cells

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 Package Contents	Catalog Number <ul style="list-style-type: none"> A14527 Buy Now A14528 Buy Now Size <ul style="list-style-type: none"> 1 vial 6 × 1 vial Concentration 1 × 10⁷ cells/vial
 Storage Conditions	<ul style="list-style-type: none"> Store in liquid nitrogen
 Required Materials	<ul style="list-style-type: none"> 125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask or other appropriate vessel for culturing suspension cells Orbital shaker in temperature and CO₂ controlled incubator Reagents and equipment to determine cell viability (e.g., hemocytometer with trypan blue or cell counter) Expi293™ Expression Medium
 Timing	Thawing and Recovery: 3–4 days Subculturing: Every 3–4 days
 Selection Guide	Protein Expression Systems Go online to view related products.
 Product Description	<ul style="list-style-type: none"> The Expi293F™ cell line is a variant of the 293 cell line, which is adapted to high-density suspension growth in Expi293™ Expression Medium.
 Important Guidelines	<ul style="list-style-type: none"> Subculture the Expi293F™ Cells a minimum of three times to allow them to recover from thawing before using them in transfection experiments. Keep cell densities between 3–5 × 10⁶ cells/mL of culture for best performance. We recommend maintaining cells in a 125-mL or 250-mL polycarbonate, disposable, sterile Erlenmeyer flask containing 25–40 mL or 50–80 mL total working volume of cell suspension, respectively. Glass flasks may be used, but clean them thoroughly after each use to avoid potential toxicity.
 Online Resources	Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support .



Protocol Outline

- Thaw cells.
- Passage cells every 3–4 days.

Expi293F™ Cell Culturing Protocol

i See page 3 to view a typical procedure for thawing and passaging Expi293F™ Cells.

Expi293F™ Cells Characteristics

Growth properties: Suspension

Doubling time: 24 hours. Doubling times may vary based on cell health, handling, and passage number.

Viability: >95% immediately after thawing. Monitor cell growth and viability the first 3–4 days to ensure the cells are not compromised. At 24 hours post-thaw, viability may drop to 80%, but should never get below 70%. By 3–4 days post-thaw, viability should be 90–95%.

Subculture conditions: Grow cells to 3–5 × 10⁶ cells/mL; then, split cells 1:10 to 0.3–0.5 × 10⁶ cells/mL every 3–4 days. Do not grow above 5 × 10⁶ cells/mL for best performance. Discard cells after passage number 40.

Scaling Up Expi293F™ Cell Culture

You can scale up the Expi293F™ cultures in spinner flasks or bioreactors. Determine the optimal spinner or impeller speed and seeding density for your culture system. We recommend that the cells be seeded at 0.3 × 10⁶ to 0.5 × 10⁶ viable cells/mL. Optimum spinner speed is approximately 100–130 rpm, and optimum impeller speed in Celligen[®] stirred tank bioreactors is 70–100 rpm.


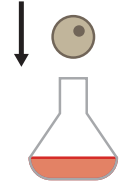
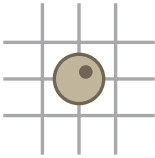

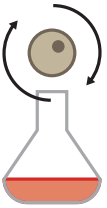
If the split ratio of cells to fresh media is less than 1:2, centrifuge the cell suspension and re-suspend the cell pellet in fresh medium before inoculating the culture.

i **Cryopreserving Expi293F™ Cells**

i **Limited Product Warranty and Disclaimer Details**

Thawing and Passaging Expi293F™ Cells

Follow the procedure below to recover and subculture Expi293F™ Cells.

	Timeline	Steps	Procedure Details		
Day 1	1 	Thaw cells	Rapidly thaw the cells in a water bath, decontaminate the vial using 70% ethanol, and open the cryovial in a class II biological cabinet.		
	2 	Add cells to medium	Add cells to 29 mL of pre-warmed medium in 125-mL shake flask.		
	3 	Count cells and determine viability	Immediately post-thaw, count cells and determine viability. Use hemocytometer and trypan blue exclusion method or automated cell counter. Cell density should be approximately 0.3×10^6 cells/mL and cell viability >90%.		
	4 	Incubate	Temperature 37°C	Humidified Atmosphere 8% CO ₂ in air	Orbital Shaker Platform 125 rpm
Days 2–4	5 	Subculture cells	<p>First passage: When cell density reaches $>1 \times 10^6$ cells/mL at $\geq 90\%$ viability (typically 2–4 days post-thaw), split cells to $0.3\text{--}0.5 \times 10^6$ cells/mL in Expi293™ medium.</p> <p>Subsequent passages: Every 3–4 days, cells should reach $3\text{--}5 \times 10^6$. Split to $0.3\text{--}0.5 \times 10^6$ cells/mL. Do not grow above 5×10^6 cells/mL.</p> <p>We recommend using a 125- or 250-mL flask containing 25–80 mL of medium, respectively.</p>		