Expi293F™ Expression System Kit

Protocol Outline
A. Thaw cells.
B. Subculture cells.
C. Transfect cells.
D. Add enhancers.
E. Generate protein or virus.

Expi293F™ Expression System Kit Characteristics
- 293F high cell density system
- Significantly higher yields
- Scalable from multi-well plates to liter scale

Expi293F™ Expression System Individual Components
The Expi293™ Expression System includes the following major components:
Click the next to each product to go to its specific protocol.

- Expi293F™ Cells: This cell line is adapted to high density, serum-free suspension culture in Expi293™ Expression Medium and is capable of producing high levels of recombinant protein.
- Expi293™ Expression Medium: This is a chemically defined, serum-free medium formulated specifically to allow high density growth and large-scale transfection of suspension Expi293F™ Cells.
- ExpiFectamine™ 293 Transfection Kit: This transfection reagent provides high transfection efficiency in suspension Expi293F™ Cells. The transfection enhancers are optimized cocktails of reagents designed to increase transient protein yields.
- Antibody Expressing Positive Control Vector: The positive control vector is provided as a positive control for transfection and expression in Expi293F™ Cells; the rabbit IgG that is produced in Expi293F™ Cells after transfection with the control vector is secreted into the Expi293™ Expression Medium.

Limited Product Warranty and Disclaimer Details

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Expi293F™ Cells

Protocol Outline

A. Thaw cells.
B. Passage cells every 3–4 days.

Expi293F™ Cell Culturing Protocol

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 × 10^6 cells/mL; then, split cells 1:10 to 0.3–0.5 × 10^6 cells/mL every 3–4 days. Do not grow above 5 × 10^6 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 × 10^6 to 0.5 × 10^6 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.

Cryopreserving Expi293F™ Cells

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# Thawing and Passaging Expi293™ Cells

Follow the procedure below to recover and subculture Expi293™ Cells.

<table>
<thead>
<tr>
<th>Timeline</th>
<th>Steps</th>
<th>Procedure Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong></td>
<td>Thaw cells</td>
<td>Rapidly thaw the cells in a water bath, decontaminate the vial using 70% ethanol, and open the cryovial in a class II biological cabinet.</td>
</tr>
<tr>
<td><strong>2</strong></td>
<td>Add cells to medium</td>
<td>Add cells to 29 mL of pre-warmed medium in 125-mL shake flask.</td>
</tr>
<tr>
<td><strong>3</strong></td>
<td>Count cells and determine viability</td>
<td>Immediately post-thaw, count cells and determine viability. Use hemocytometer and trypan blue exclusion method or automated cell counter. Cell density should be approximately $0.3 \times 10^6$ cells/mL and cell viability $&gt; 90%$.</td>
</tr>
<tr>
<td><strong>4</strong></td>
<td>Incubate</td>
<td><strong>Temperature</strong> 37°C <strong>Humidified Atmosphere</strong> 8% CO$_2$ in air <strong>Orbital Shaker Platform</strong> 125 rpm</td>
</tr>
</tbody>
</table>
| **5**    | Subculture cells | **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 $0.3–0.5 \times 10^6$ cells/mL in Expi293™ medium.  
**Subsequent passages:** Every 3–4 days, cells should reach $3–5 \times 10^6$. Split to $0.3–0.5 \times 10^6$ cells/mL. Do not grow above $5 \times 10^6$ cells/mL. We recommend using a 125- or 250-mL flask containing 25–80 mL of medium, respectively. |

For support, visit [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).
Expi293™ Expression Medium

Protocol Outline

A. Thaw cells.
B. Passage cells every 3–4 days.

Expi293F™ Cell Culturing Protocol

See page 5 to view a typical thawing and culturing procedure.

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 0.3–0.5 × 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.

Adapting FreeStyle™ 293-F Cells to Expi293™ Expression Medium

Cryopreserving Expi293F™ Cells.

Limited Product Warranty and Disclaimer Details
# Thawing and Culturing Expi293F™ Cells in Expi293™ Medium

Follow the procedure below to thaw and passage Expi293F™ Cells in Expi293™ Expression Medium.

<table>
<thead>
<tr>
<th>Timeline</th>
<th>Steps</th>
<th>Procedure Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Thaw cells</td>
<td>Rapidly thaw the cells in a water bath, decontaminate the vial using 70% ethanol, and open the cryovial in a class II biological cabinet.</td>
</tr>
<tr>
<td></td>
<td>Add cells to medium</td>
<td>Add cells to 29 mL of pre-warmed medium in 125-mL shake flask.</td>
</tr>
<tr>
<td></td>
<td>Count cells and determine viability</td>
<td>Immediately post-thaw, count cells and determine viability. Use hemocytometer and trypan blue exclusion method or automated cell counter. Cell density should be approximately $0.3 \times 10^6$ cells/mL and cell viability $&gt;90%$.</td>
</tr>
</tbody>
</table>
| Days 2–4 | Incubate | **Temperature** 37°C  
**Humidified Atmosphere** 8% CO$_2$ in air  
**Orbital Shaker Platform** 125 rpm |
|          | Subculture cells | **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 $0.3 \times 10^6$ cells/mL in Expi293™ medium.  
**Subsequent passages**: Every 3–4 days, cells should reach 3–5 $\times 10^6$. Split to 0.3–0.5 $\times 10^6$ cells/mL. Do not grow above 5 $\times 10^6$ cells/mL. We recommend using a 125- or 250-mL flask containing 25–80 mL of medium, respectively. |

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Protocol Outline

A. Culture cells for at least four passages after thawing.
B. Prepare and add lipid-DNA complexes to cells.
C. Add enhancers.
D. Incubate cells for 1–7 days.
E. Harvest.

ExpiFectamine™ 293 Transfection Kit Protocol

See page 7 to view a typical transfection procedure.

Transfection Conditions for Expi293F™ Cells

For each 30-mL transfection, use 7.5 × 10^7 cells in 25.5 mL of Expi293™ Expression Medium. Scale your transfections up or down by proportionately adjusting the amounts of the reagents used.

- Final transfection volume: 30 mL
- Number of cells to transfect: 7.5 × 10^7 cells with >95% viability (final cell density of 2.5 × 10^6 cells/mL)
- Amount of plasmid DNA: 30 μg. Use 1 μg of DNA for every mL of transfection reaction.
- Amount of ExpiFectamine™ 293 Reagent: 81 μL. Use 2.7 μL ExpiFectamine™ 293 Reagent per 1 μg of plasmid DNA transfected.

Scaling Up or Down Transfections

Optimization for Other 293 Cells

If you are using 293 cells other than Expi293™F Cells, optimize the transfection conditions by varying the amount of Expi293™F Cells (e.g., 40, 50, 60, 80, 100 μL) used with 30 μg plasmid DNA.

- Final transfection volume: 30 mL
- Number of cells to transfect: 7.5 × 10^7 cells (final cell density of 2.5 × 10^6 cells/mL) with >95% viability.
- Amount of plasmid DNA: 30 μg
- Amount of ExpiFectamine™ 293 Reagent: 40–100 μL. Use 2.7 μL ExpiFectamine™ 293 Reagent per 1 μg of plasmid DNA transfected.

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Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

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Transfecting Expi293™F Cells

Transfect Expi293™F cells according to the table below.

<table>
<thead>
<tr>
<th>Timeline</th>
<th>Steps</th>
<th>Procedure Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day -1</td>
<td>1. Prepare cells</td>
<td>Seed $6 \times 10^7$ viable cells in 30 mL of Expi293™ Expression Medium. For each 30-mL transfection, you will need $7.5 \times 10^7$ cells/mL.</td>
</tr>
<tr>
<td></td>
<td>2. Incubate cells</td>
<td>Use a hemocytometer and trypan blue dye exclusion or automated cell counter to determine cell number and viability. The cell density should be $3-5 \times 10^6$ cells/mL. To proceed, cell viability must be $&gt;95%$. Cell density of $&lt;3 \times 10^6$ cells/mL or $&lt;95%$ viability will result in a loss in performance.</td>
</tr>
<tr>
<td></td>
<td>3. Count cells and determine viability</td>
<td>Add $7.5 \times 10^7$ cells to 25.5 mL of Expi293™ Expression Medium ($2.9 \times 10^6$ cells/mL) in a 125-mL flask.</td>
</tr>
<tr>
<td></td>
<td>4. Dilute cells</td>
<td>For each 30-mL transfection, prepare as follows:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a. Dilute 30 μg of plasmid DNA in Opti-MEM® I Reduced Serum Medium to a total volume of 1.5 mL. Mix gently.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. Dilute 81 μL of ExpiFectamine™ 293 Reagent in Opti-MEM® I medium to a total volume of 1.5 mL. Mix gently and incubate for 5 minutes at room temperature (longer incubation times may result in decreased activity).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c. After the 5-minute incubation, add the diluted DNA to the diluted ExpiFectamine™ 293 Reagent to obtain a total volume of 3 mL. Mix gently.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d. Incubate the mixture for 20 minutes at room temperature to allow the DNA-ExpiFectamine™ 293 Reagent complexes to form.</td>
</tr>
<tr>
<td></td>
<td>5. Prepare lipid-DNA complexes</td>
<td>Add 3 mL of complex to each flask. Each flask should contain 28.5 mL.</td>
</tr>
<tr>
<td></td>
<td>6. Add DNA-lipid complexes to cells</td>
<td>After incubating cells for 20 hours, add 150 μL of ExpiFectamine™ 293 Transfection Enhancer 1 and 1.5 mL of ExpiFectamine™ 293 Transfection Enhancer 2 to each flask. (Enhancers 1 and 2 can be combined prior to addition to the cell culture.) The final volume should be approximately 30 mL in each 125-mL flask.</td>
</tr>
<tr>
<td></td>
<td>7. Incubate cells</td>
<td>Time for optimal protein expression depends on the nature of your recombinant protein. Harvest media if recombinant protein is secreted. Assay for recombinant protein expression.</td>
</tr>
<tr>
<td></td>
<td>8. Add enhancers</td>
<td>For support, visit <a href="http://www.lifetechnologies.com/support">www.lifetechnologies.com/support</a>.</td>
</tr>
<tr>
<td>Days 1–7</td>
<td>9. Harvest cells or media</td>
<td>For support, visit <a href="http://www.lifetechnologies.com/support">www.lifetechnologies.com/support</a>.</td>
</tr>
</tbody>
</table>