


# Expi293F™ Expression System Kit

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**Catalog Number** A14635

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 **Quantities**


## Package Contents

- Expi293F™ Cells
- ExpiFectamine™ 293 Transfection Kit
- Expi293™ Expression Medium
- Opti-MEM® I Reduced-Serum Medium
- Antibody Expressing Positive Control Vector



## Storage Conditions

- Store cells in liquid nitrogen.
- Store reagent, enhancers, and media at 2°C to 8°C.
- Protect enhancers and media from light.
- Store the control vector at -20°C.



## Required Materials

- 125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask or other appropriate vessel for culturing suspension cells
- Orbital shaker in temperature and CO<sub>2</sub> controlled incubator



## Timing

Thawing and Recovery: 2–4 days  
 Subculturing: Every 3–4 days  
 Transfecting: 1–7 days



## Selection Guide

[Protein Expression Systems](#)

Go online to view related products.





## Product Description

- The Expi293™ Expression System facilitates large-scale transfection of suspension 293 human embryonic kidney cells in a defined, serum-free medium for expression of proteins and virus.
- Transfection and expression experiments may be performed directly in Expi293™ Expression Medium without the need for media change.
- The kit provides cells, culture medium, and reagents to transfect 1 liter of cell culture and yields 250 mg/L of protein with supplied antibody positive control.
- This kit is not an animal origin-free (AOF) product.
- Keep cell densities between 3–5 × 10<sup>6</sup> cells/mL of culture for best performance.



## Important Guidelines

-  General Cell Handling
-  Preparing Media



## Online Resources

Visit our [product page](#) for additional information and protocols. For support, visit [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).



## Protocol Outline


- Thaw cells.
- Subculture cells.
- Transfect cells.
- Add enhancers.
- 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

# Expi293F™ Cells

[Learn More](#)

 <b>Package Contents</b>	<b>Catalog Number</b> <ul style="list-style-type: none"> <li>A14527 <a href="#">Buy Now</a></li> <li>A14528 <a href="#">Buy Now</a></li> </ul> <b>Size</b> <ul style="list-style-type: none"> <li>1 vial</li> <li>6 × 1 vial</li> <li>Concentration 1 × 10<sup>7</sup> cells/vial</li> </ul>
 <b>Storage Conditions</b>	<ul style="list-style-type: none"> <li>Store in liquid nitrogen</li> </ul>
 <b>Required Materials</b>	<ul style="list-style-type: none"> <li>125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask or other appropriate vessel for culturing suspension cells</li> <li>Orbital shaker in temperature and CO<sub>2</sub> controlled incubator</li> <li>Reagents and equipment to determine cell viability (e.g., hemocytometer with trypan blue or cell counter)</li> <li>Expi293™ Expression Medium</li> </ul>
 <b>Timing</b>	Thawing and Recovery: 3–4 days Subculturing: Every 3–4 days
 <b>Selection Guide</b>	<a href="#">Protein Expression Systems</a> Go online to view related products.
 <b>Product Description</b>	<ul style="list-style-type: none"> <li>The Expi293F™ cell line is a variant of the 293 cell line, which is adapted to high-density suspension growth in Expi293™ Expression Medium.</li> </ul>
 <b>Important Guidelines</b>	<ul style="list-style-type: none"> <li>Subculture the Expi293F™ Cells a minimum of three times to allow them to recover from thawing before using them in transfection experiments.</li> <li>Keep cell densities between 3–5 × 10<sup>6</sup> cells/mL of culture for best performance.</li> <li>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.</li> <li>Glass flasks may be used, but clean them thoroughly after each use to avoid potential toxicity.</li> </ul>
 <b>Online Resources</b>	Visit our <a href="#">product page</a> for additional information and protocols. For support, visit <a href="http://www.lifetechnologies.com/support">www.lifetechnologies.com/support</a> .



## 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 × 10<sup>6</sup> cells/mL; then, split cells 1:10 to 0.3–0.5 × 10<sup>6</sup> cells/mL every 3–4 days. Do not grow above 5 × 10<sup>6</sup> 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<sup>6</sup> to 0.5 × 10<sup>6</sup> viable cells/mL. Optimum spinner speed is approximately 100–130 rpm, and optimum impeller speed in Celligen<sup>®</sup> stirred tank bioreactors is 70–100 rpm.


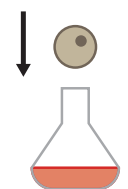
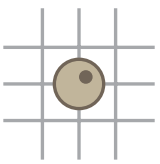

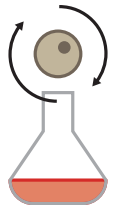
If the split ratio of cells to fresh media is less than 1:2, centrifuge the cell suspension and re-suspend the cell pellet in fresh medium before inoculating the culture.

**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	 <b>Thaw cells</b>	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	 <b>Add cells to medium</b>	Add cells to 29 mL of pre-warmed medium in 125-mL shake flask.		
	3	 <b>Count cells and determine viability</b>	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 >90%.		
	4	 <b>Incubate</b>	<b>Temperature</b> 37°C	<b>Humidified Atmosphere</b> 8% CO <sub>2</sub> in air	<b>Orbital Shaker Platform</b> 125 rpm
Days 2–4	5	 <b>Subculture cells</b>	<p><b>First passage:</b> When cell density reaches <math>&gt;1 \times 10^6</math> cells/mL at <math>\geq 90\%</math> viability (typically 2–4 days post-thaw), split cells to <math>0.3\text{--}0.5 \times 10^6</math> cells/mL in Expi293™ medium.</p> <p><b>Subsequent passages:</b> Every 3–4 days, cells should reach <math>3\text{--}5 \times 10^6</math>. Split to <math>0.3\text{--}0.5 \times 10^6</math> cells/mL. Do not grow above <math>5 \times 10^6</math> cells/mL.</p> <p>We recommend using a 125- or 250-mL flask containing 25–80 mL of medium, respectively.</p>		

# Expi293™ Expression Medium [Learn More](#)


	<b>Package Contents</b>	<b>Catalog Number</b>	<b>Size</b>
		<ul style="list-style-type: none"> <li>A14351-01 <a href="#">Buy Now</a></li> <li>A14351-02 <a href="#">Buy Now</a></li> </ul>	1000 mL 6 × 1000 mL


	<b>Storage Conditions</b>	<ul style="list-style-type: none"> <li>Store at 2°C to 8°C for a 12-month shelf life.</li> </ul>
		<b>IMPORTANT!</b> Protect from light.


	<b>Required Materials</b>	<ul style="list-style-type: none"> <li>Expi293F™ Cells</li> <li>125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask or other appropriate vessel for culturing suspension cells</li> <li>Orbital shaker in temperature and CO<sub>2</sub> controlled incubator</li> <li>Reagents and equipment to determine cell viability (e.g., hemocytometer with trypan blue or cell counter)</li> </ul>
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	<b>Timing</b>	Thawing and Recovery: 3–4 days Subculturing: Every 3–4 days
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	<b>Selection Guide</b>	<a href="#">Protein Expression Systems</a> Go online to view related products.
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	<b>Product Description</b>	<ul style="list-style-type: none"> <li>Expi293™ Expression Medium is an optimized, chemically defined formulation designed to support the high-density culture and transfection of Expi293F™ Cells in suspension.</li> <li>Other 293 cell lines (e.g., FreeStyle™ 293-F Cells) can be used with adaptation. This medium is not recommended for adherent 293 cell culture.</li> <li>This medium does not contain any protein, undefined lysates, or components of animal origin.</li> </ul>
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	<b>Important Guidelines</b>	<ul style="list-style-type: none"> <li>Expi293™ Expression Medium contains GlutaMAX™-I reagent and does not require supplementation with L-glutamine or GlutaMAX™-I reagent.</li> <li>Subculture Expi293F™ Cells when they reach a density of approximately <math>3 \times 10^6</math> to <math>5 \times 10^6</math> viable cells/mL, typically every 3–4 days. Split the culture to <math>0.3 \times 10^6</math>–<math>0.5 \times 10^6</math> cells/mL.</li> <li>Keep cell densities between <math>3</math>–<math>5 \times 10^6</math> cells/mL of culture for best performance.</li> </ul>
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	<b>Online Resources</b>	Visit our <a href="#">product page</a> for additional information and protocols. For support, visit <a href="http://www.lifetechnologies.com/support">www.lifetechnologies.com/support</a> .
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## Protocol Outline

- Thaw cells.
- 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  $0.3$ – $0.5 \times 10^6$  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.


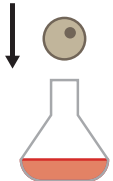
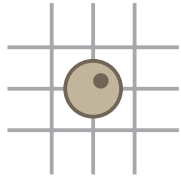


## Adapting FreeStyle™ 293-F Cells to Expi293™ Expression Medium

## 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.

	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 $0.3 \times 10^6$ cells/mL and cell viability >90%.		
	4 	Incubate	Temperature 37°C	Humidified Atmosphere 8% CO <sub>2</sub> in air	Orbital Shaker Platform 125 rpm
Days 2-4	5 	Subculture cells	<p><b>First passage:</b> When cell density reaches <math>&gt;1 \times 10^6</math> cells/mL at <math>\geq 90\%</math> viability (typically 2–4 days post-thaw), split cells to <math>0.3 \times 10^6</math> cells/mL in Expi293™ medium.</p> <p><b>Subsequent passages:</b> Every 3–4 days, cells should reach <math>3\text{--}5 \times 10^6</math>. Split to <math>0.3\text{--}0.5 \times 10^6</math> cells/mL. Do not grow above <math>5 \times 10^6</math> cells/mL. We recommend using a 125- or 250-mL flask containing 25–80 mL of medium, respectively.</p>		

# ExpiFectamine™ 293 Transfection Kit




## Package Contents

Catalog Number	Volume	Enough to Transfect
A14524 <a href="#">Buy Now</a>	2.7 mL	1 L culture
A14525 <a href="#">Buy Now</a>	27 mL	10 L culture
A14526 <a href="#">Buy Now</a>	135 mL	50 L culture

- ExpiFectamine™ 293 Reagent
- ExpiFectamine™ 293 Transfection Enhancer 1
- ExpiFectamine™ 293 Transfection Enhancer 2



## Storage Conditions

- Store at 2°C to 8°C.



## Required Materials

- Plasmid DNA
- Orbital shaker in temperature and CO<sub>2</sub> controlled incubator
- 125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask
- Antibody Expressing Positive Control Vector
- Expi293™ or 293 Cells
- Expi293™ Expression Medium
- Opti-MEM® I Reduced Serum Medium



## Timing

Preparation: 1.5 hours  
Transfection: 1–7 days



## Selection Guide

[Protein Expression Systems](#)  
Go online to view related products.



## Product Description

- ExpiFectamine™ 293 Reagent is a proprietary cationic lipid-based reagent for transfecting nucleic acids into eukaryotic cells.



## Important Guidelines

- Subculture the Expi293F™ Cells a minimum of three times to allow them to recover from thawing before using them in transfection experiments.
- Calculate the number of cells needed for your transfections, and expand the cells accordingly.
- Include positive and negative controls.
- Plasmid DNA must be clean, sterile, and free from phenol and sodium chloride. We recommend isolating plasmid DNA using a Purelink® HiPure Plasmid Kit.
- Gently mix the ExpiFectamine™ 293 Reagent by pipetting it up and down before use.



## Online Resources


Visit our [product page](#) for additional information and protocols. For support, visit [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).



## Protocol Outline

- Culture cells for at least four passages after thawing.
- Prepare and add lipid-DNA complexes to cells.
- Add enhancers.
- Incubate cells for 1–7 days.
- 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  $7.5 \times 10^7$  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:  $7.5 \times 10^7$  cells with >95% viability (final cell density of  $2.5 \times 10^6$  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 ExpiFectamine™ 293 Reagent (e.g., 40, 50, 60, 80, 100 µL) used with 30 µg plasmid DNA.

- Final transfection volume: 30 mL
- Number of cells to transfect:  $7.5 \times 10^7$  cells (final cell density of  $2.5 \times 10^6$  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.

## Limited Product Warranty and Disclaimer Details

## Transfecting Expi293F™ Cells

Transfect Expi293™F cells according to the table below.

Timeline		Steps	Procedure Details		
Day -1	1	 <b>Prepare cells</b>	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.		
	2	 <b>Incubate cells</b>	<b>Temperature</b> 37°C	<b>Humidified atmosphere</b> 8% CO <sub>2</sub> in air	<b>Orbital shaker platform</b> 125 rpm
Day 0	3	 <b>Count cells and determine viability</b>	Use a hemocytometer and trypan blue dye exclusion or automated cell counter to determine cell number and viability. The cell density should be $3\text{--}5 \times 10^6$ cells/mL. To proceed, cell viability must be >95%. Cell density of $<3 \times 10^6$ cells/mL or <95% viability will result in a loss in performance.		
	4	 <b>Dilute cells</b>	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.		
	5	 <b>Prepare lipid-DNA complexes</b>	For each 30-mL transfection, prepare as follows: <ol style="list-style-type: none"> <li>Dilute 30 µg of plasmid DNA in Opti-MEM® I Reduced Serum Medium to a total volume of 1.5 mL. Mix gently.</li> <li>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).</li> <li>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.</li> <li>Incubate the mixture for 20 minutes at room temperature to allow the DNA-ExpiFectamine™ 293 Reagent complexes to form.</li> </ol>		
Days 1-7	6	 <b>Add DNA-lipid complexes to cells</b>	Add 3 mL of complex to each flask. Each flask should contain 28.5 mL.		
	7	 <b>Incubate cells</b>	<b>Temperature</b> 37°C	<b>Humidified atmosphere</b> 8% CO <sub>2</sub> in air	<b>Orbital shaker platform</b> 125 rpm
Days 1-7	8	 <b>Add enhancers</b>	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.		
	9	 <b>Harvest cells or media</b>	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.		