Expi293F™ Expression System Kit

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Catalog Number A14635 Buy Now

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_2 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 $\times 10^6$ 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.

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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.

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Expi293F™ Cells

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Package Contents

Catalog Number		Size
A14527	Buy Now	1 vial
A14528	Buy Now	6 × 1 vial

- Concentration 1 × 10^7 cells/vial

Storage Conditions

- Store in liquid nitrogen

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_2 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

- 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

- 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 × 10^6 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

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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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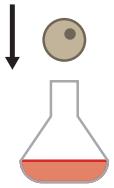
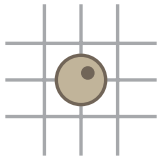
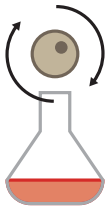
Thawing and Passaging Expi293F™ Cells

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**	**First passage:** When cell density reaches >1 × 10^6 cells/mL at ≥ 90% viability (typically 2–4 days post-thaw), split cells to 0.3–0.5 × 10^6 cells/mL in Expi293™ medium. **Subsequent passages:** Every 3–4 days, cells should reach 3–5 × 10^6. Split to 0.3–0.5 × 10^6 cells/mL. Do not grow above 5 × 10^6 cells/mL. We recommend using a 125- or 250-mL flask containing 25–80 mL of medium, respectively.		

Expi293™ Expression Medium

Learn More

Package Contents

Catalog Number	Size
A14351-01 Buy Now	1000 mL
A14351-02 Buy Now	6 × 1000 mL

Storage Conditions

- Store at 2°C to 8°C for a 12-month shelf life.

IMPORTANT! Protect from light.

Required Materials

- Expi293F™ Cells
- 125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask or other appropriate vessel for culturing suspension cells
- Orbital shaker in temperature and CO_2 controlled incubator
- Reagents and equipment to determine cell viability (e.g., hemocytometer with trypan blue or cell counter)

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

- Expi293™ Expression Medium is an optimized, chemically defined formulation designed to support the high-density culture and transfection of Expi293F™ Cells in suspension.
- 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.
- This medium does not contain any protein, undefined lysates, or components of animal origin.

Important Guidelines

- Expi293™ Expression Medium contains GlutaMAX™-I reagent and does not require supplementation with L-glutamine or GlutaMAX™-I reagent.
- Subculture Expi293F™ Cells when they reach a density of approximately 3×10^6 to 5×10^6 viable cells/mL, typically every 3–4 days. Split the culture to 0.3×10^6–0.5×10^6 cells/mL.
- Keep cell densities between $3–5 \times 10^6$ cells/mL of culture for best performance.

Online Resources

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

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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 $0.3–0.5 \times 10^6$ 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.

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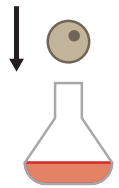
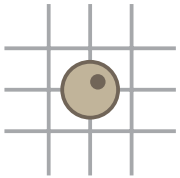
Thawing and Culturing Expi293F™ Cells in Expi293™ Medium

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 × 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**	**First passage:** When cell density reaches >1 × 10^6 cells/mL at ≥ 90% viability (typically 2–4 days post-thaw), split cells to 0.3 × 10^6 cells/mL in Expi293™ medium. **Subsequent passages:** Every 3–4 days, cells should reach 3–5 × 10^6. Split to 0.3–0.5 × 10^6 cells/mL. Do not grow above 5 × 10^6 cells/mL. We recommend using a 125- or 250-mL flask containing 25–80 mL of medium, respectively.

ExpiFectamine™ 293 Transfection Kit

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Catalog Number	Volume	Enough to Transfect
A14524 Buy Now	2.7 mL	1 L culture
A14525 Buy Now	27 mL	10 L culture
A14526 Buy Now	135 mL	50 L culture

Package Contents

- 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_2 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.

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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 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: 7.5×10^7 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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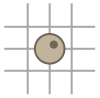
Transfecting Expi293F™ Cells

Transfect Expi293™F cells according to the table below.

Timeline		Steps	Procedure Details
Day –1	1 	**Prepare cells**	Seed 6 × 10^7 viable cells in 30 mL of Expi293™ Expression Medium. For each 30-mL transfection, you will need 7.5 × 10^7 cells/mL.
	2 	**Incubate cells**	**Temperature** 37°C; **Humidified atmosphere** 8% CO_2 in air; **Orbital shaker platform** 125 rpm
Day 0	3 	**Count cells and determine viability**	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 × 10^6 cells/mL. To proceed, cell viability must be >95%. Cell density of <3 × 10^6 cells/mL or <95% viability will result in a loss in performance.
	4 	**Dilute cells**	Add 7.5 × 10^7 cells to 25.5 mL of Expi293™ Expression Medium (2.9 × 10^6 cells/mL) in a 125-mL flask.
	5 	**Prepare lipid-DNA complexes**	For each 30-mL transfection, prepare as follows: a. Dilute 30 µg of plasmid DNA in Opti-MEM® I Reduced Serum Medium to a total volume of 1.5 mL. Mix gently. 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). 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. d. Incubate the mixture for 20 minutes at room temperature to allow the DNA-ExpiFectamine™ 293 Reagent complexes to form.
	6 	**Add DNA-lipid complexes to cells**	Add 3 mL of complex to each flask. Each flask should contain 28.5 mL.
	7 	**Incubate cells**	**Temperature** 37°C; **Humidified atmosphere** 8% CO_2 in air; **Orbital shaker platform** 125 rpm
Days 1–7	8 	**Add enhancers**	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 	**Harvest cells or media**	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.