Expi293F™ Expression System Kit

gibco by life technologies™

Print Options

Learn More

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.

For Research Use Only. Not for use in diagnostic procedures.

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

life technologies™

Expi293F™ Cells

Learn More

gibco by life technologies™

Print Options

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

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

For Research Use Only. Not for use in diagnostic procedures.

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.

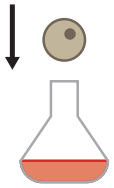
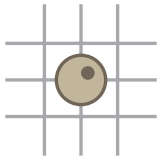
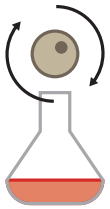
Cryopreserving Expi293F™ Cells

Limited Product Warranty and Disclaimer Details

life technologies™

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 × 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.

For Research Use Only. Not for use in diagnostic procedures.

gibco by life technologies™

Print Options

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 × 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

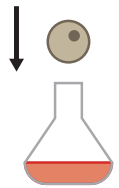
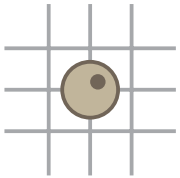
Cryopreserving Expi293F™ Cells.

Limited Product Warranty and Disclaimer Details

life technologies™

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

gibco by life technologies™

Print Options

Learn More

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.

For Research Use Only. Not for use in diagnostic procedures.

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.

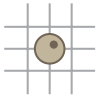
- Final transfection volume: 30 mL
- Number of cells to transfect: 7.5×10^7 cells (final cell density of 2.5×10^6 cells/mL) with >95% viability.
- Amount of plasmid DNA: 30 µg
- Amount of ExpiFectamine™ 293 Reagent: 40–100 µL.
  Use 2.7 µL ExpiFectamine™ 293 Reagent per 1 µg of plasmid DNA transfected.

Limited Product Warranty and Disclaimer Details

life technologies™

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.