USER GUIDE

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Expi293™ MembranePro™ Expression System

For expression of functional membrane proteins in Expi293F™ cells using virus-like particles

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Contents

Kit contents and storage

Types of kits

Product	Amount	Cat. no.
Expi293™ MembranePro™ Expression System	10 reactions	A25869
	10 ×10 reactions	A25870

Kit contents

Each product contains the following components. Upon receipt, store each component as detailed below.

Component	Size	Quantity	Storage conditions
Expi293™ MembranePro™ Expression System – 10 reactions (Cat. no. A25869)			
MembranePro™ Reagent	300 µg/tube	1 tube	–20°C
MembranePro™ Precipitation Mix	75 mL/bottle	1 bottle	Room temperature
ExpiFectamine™ 293 Reagent	2.7 mL/bottle	1 bottle	2°C to 8°C
ExpiFectamine™ 293 Transfection Enhancer 1	5 mL/bottle	1 bottle	2°C to 8°C
ExpiFectamine™ 293 Transfection Enhancer 2	50 mL/bottle	1 bottle	2°C to 8°C, Protect from light
Opti-MEM® I Reduced-Serum Medium	100 mL/bottle	1 bottle	2°C to 8°C, Protect from light
Expi293™ Expression Medium	1 L/bottle	1 bottle	2°C to 8°C, Protect from light
Expi293™ MembranePro™ Expression System – 10 ×10 reactions (Cat. no. A25870)			
MembranePro™ Reagent	300 µg/tube	10 tubes	–20°C
MembranePro™ Precipitation Mix	75 mL/bottle	10 bottles	Room temperature
ExpiFectamine™ 293 Reagent	2.7 mL/bottle	10 bottles	2°C to 8°C
ExpiFectamine™ 293 Transfection Enhancer 1	5 mL/bottle	10 bottles	2°C to 8°C
ExpiFectamine™ 293 Transfection Enhancer 2	50 mL/bottle	10 bottles	2°C to 8°C, Protect from light
Opti-MEM® I Reduced-Serum Medium	100 mL/bottle	10 bottles	2°C to 8°C, Protect from light
Expi293™ Expression Medium	1 L/bottle	10 bottles	2°C to 8°C, Protect from light

Components of Expi293™ MembranePro™ Expression System

- **MembranePro™ Reagent:** This reagent is optimized for high-yield packaging and secretion of virus-like particles (VLPs) that contain the functional membrane protein of interest into the extracellular medium.
- **MembranePro™ Precipitation Mix:** This mix is for harvesting VLPs released into the growth medium at clinical centrifuge speeds, reducing the mechanical damage to the particles by strong shear forces encountered during ultracentrifugation.
- **ExpiFectamine™ 293 Reagent:** This transfection reagent provides high transfection efficiency in suspension Expi293F™ Cells.
- **ExpiFectamine™ 293 Transfection Enhancers 1 and 2:** The transfection enhancers are optimized cocktails of reagents designed to increase transient protein yields.
- **Opti-MEM® I Reduced-Serum Medium:** This medium facilitates the optimal formation of DNA-MembranePRO™ Reagent-ExpiFectamine™ 293 Reagent complexes.
- **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.

Materials required but not supplied

Item	Catalog no.
Expi293F™ Cells	A14527
pEF6 V5-His TOPO® Expression Kit	K9610-20

Note: The Expi293™ MembranePro™ Expression System protocol and media recommendations are optimized for use with Expi293F™ Cells for the production of MembranePro™ particles. They may deviate slightly from the recommendations made in the Expi293F™ Cells User Guide. For optimal results, follow the media and culture protocol for MembranePro™ particle production specified in this user guide.

CAUTION! As with other human cell lines, when working with Expi293F™ Cells, handle as potentially biohazardous material under at least Biosafety Level-2 (BL-2) containment. For more information on BL-2 guidelines, refer to *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed., published by the Centers for Disease Control, which is available for downloading at:

www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Safety Data Sheet (SDS) before handling.

Description of the system

Expi293™ MembranePro™ Expression System

Expi293™ MembranePro™ Expression technology

The Expi293™ MembranePro™ Expression technology is a system for the expression and display of mammalian cell surface membrane proteins, including G-protein coupled receptors (GPCRs), in an aqueous-soluble format. The system uses virus-like particles (VLPs) to capture lipid raft regions of the cell's plasma membrane as the VLPs are secreted from the cell. Using this system, it is possible to capture and display endogenous or overexpressed GPCRs and other cell surface membrane proteins in their native context for downstream assays. Because the VLPs are packaged by the cell and secreted into the culture medium, VLPs allow the isolation of functional membrane proteins by simply decanting and clarifying the culture medium, and isolating the VLPs by precipitation. This represents a substantial savings in time, effort, and required machinery over preparing cell membrane fractions. Because VLPs capture receptor-rich regions of the plasma membrane, your GPCR may also be substantially enriched over crude membrane preparations.

How Expi293™ MembranePro™ Expression works

Virus-like particles (VLPs) are subviral particles that self-assemble from virus-derived core structural proteins (gag proteins). Because VLPs lack the proteinaceous and nucleic acid components of their viral counterparts, they are noninfectious.

The Expi293™ MembranePro™ Expression System takes advantage of the functionality of the lentiviral gag protein, which, when expressed in Expi293F™ Cells, travels to the plasma membrane where it forms buds underneath the lipid rafts. Because lipid rafts play an active role in regulating the conformational state and dynamic sorting of membrane proteins, recombinant and endogenous GPCRs and other receptors are localized in these microdomains after having passed the cellular quality control. As the VLP buds from the cell, it becomes enveloped in this portion of the plasma membrane and captures the membrane proteins in their native context.

By capturing just the membrane protein-rich lipid rafts, this versatile and ready-to-use system distinguishes itself from crude membrane fractions, which contain total plasma membrane as well as contaminating amounts of endoplasmic reticulum, Golgi apparatus, and nuclear envelope.

CAUTION! Although the Expi293™ MembranePro™ Expression System does not create actual viral particles, resultant VLPs may still pose some biohazardous risk if fusogenic particles come in contact with bare skin. If used with cells containing or expressing viral genomic sequences, you may produce transducing-capable VLPs. Observe Risk Group 2 (RG-2) guidelines for handling and disposing of biohazardous materials.
For more information on RF-2 guidelines, refer to NIH Guidelines for Research Involving Recombinant DNA Molecules, which is available for downloading at **http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.pdf**

Components of Expi293™ MembranePro™ Expression System

The Expi293™ MembranePro™ Expression System includes the reagents for functional expression of membrane proteins but does not contain the expression vector or the Expi293F™ Cells. The Expi293™ MembranePro™ Expression System is available in two sizes, allowing 10 or 100 reactions. It contains the components listed below.

- MembranePro™ Reagent
- MembranePro™ Precipitation Mix
- ExpiFectamine™ 293 Reagent
- ExpiFectamine™ 293 Transfection Enhancers 1 and 2
- Opti-MEM® I Reduced-Serum Medium
- Expi293™ Expression Medium

A detailed description of each component is listed below.

MembranePro™ Reagent

This reagent is optimized for high-yield packaging and secretion of virus-like particles (VLPs) that contain the functional membrane protein of interest into the extracellular medium.

MembranePro™ Precipation Mix

This mix is for harvesting VLPs released into the growth medium at clinical centrifuge speeds, reducing the mechanical damage to the particles by strong shear forces encountered during ultracentrifugation.

ExpiFectamine™ 293 Reagent

ExpiFectamine™ 293 Reagent is a proprietary formulation suitable for transfection of nucleic acids into eukaryotic cells. The use of the ExpiFectamine™ 293 Reagent to transfect Expi293F™ Cells provides the following advantages:

- ExpiFectamine™ 293 Reagent demonstrates high transfection efficiency in high-density suspension Expi293F™ Cells (cultured in Expi293™ Expression Medium).
- DNA-ExpiFectamine™ 293 Reagent complexes can be added directly to cells in culture medium.
- It is not necessary to remove complexes or change or add medium following transfection.

ExpiFectamine™ 293 Reagent is also available separately as part of the ExpiFectamine™ 293 Transfection Kit (see page 24 for ordering information). For more information, see our website (**www.lifetechnologies.com**) or contact Technical Support (see page 25).

ExpiFectamine™ 293 Transfection Enhancers 1 and 2

The transfection enhancers are optimized cocktails of reagents designed to increase transient protein yields.

ExpiFectamine™ 293 Transfection Enhancers 1 and 2 are also available separately as part of the ExpiFectamine™ 293 Transfection Kit (see page 24 for ordering information. For more information, see our website (**www.lifetechnologies.com**) or contact Technical Support (see page 25).

Opti-MEM® I Reduced-Serum Medium

Opti-MEM® I Reduced-Serum Medium facilitates the optimal formation of DNA-MembrandPRO™ Reagent-ExpiFectamine™ 293 Reagent complexes. Opti-MEM® I Reduced Serum Medium is buffered with HEPES and sodium bicarbonate, and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, and growth factors. The protein level is minimal (15 µg/mL) with insulin

and transferrin being the only protein supplements. Phenol red is included at a reduced concentration as a pH indicator.

Opti-MEM® I Reduced-Serum Medium is also available separately (see page 24 for ordering information). For more information, see our website (**www.lifetechnologies.com**) or contact Technical Support (see page 25).

Expi293™ Expression Medium

Expi293™ Expression Medium is an optimized, chemically defined formulation designed to support the high-density culture and transfection of 293 cells (e.g., Expi293F™ Cells) in suspension. This chemically defined medium does not contain any protein, undefined lysates, or components of animal origin. Expi293™ Expression Medium is a complete, ready-to-use medium formulated with GlutaMAX™-I reagent, and it requires no supplementation. Expi293™ Expression Medium is also available separately (see page 24 for ordering information). For more information, see our website (**www.lifetechnologies.com**) or contact Technical Support (see page 25).

Advantages of Expi293™ MembranePro™ Expression

- Allows convenient over-expression and display of functional membrane proteins, including GPCRs. Depending on the efficiency of expression of your GPCR and its affinity for your test ligand, a single reaction may generate sufficient VLP sample for up to 1000 ligand binding assay data points.
- Displays membrane proteins on lipoprotein particles released into the growth medium, allowing easy harvest.
- Enriches for receptor-specific activity by capturing membrane protein-rich lipid rafts.
- Does not require harsh treatments or detergents, which can inactivate membrane proteins.
- Does not require ultracentrifugation steps for clarifying the medium, where strong shear forces can damage the VLPs.
- Obviates the need to establish stable cell lines for protein expression, which carry the risk of down-regulation of expression due to protein toxicity.
- Get more than a 20-fold increase in membrane protein production compared to the adherent MembranePro™ Functional Protein Expression System.
- Ease of use of suspension culture makes the process less labor intensive
- There is the ability to scale production without the need for multiple flasks.

Possible applications

VLPs produced using the Expi293™ MembranePro™ Expression System can substitute for membrane fractions in a variety of downstream applications. These may include ligand binding experiments or other functional or biochemical assays.

Note: There are many factors that affect protein yield, solubility, and function. Therefore, your expressed membrane protein might **not** be suitable for all the downstream applications listed above.

Note: You can use the Expi293™ MembranePro™ Expression System to express, package into, and display on VLPs most membrane proteins that are destined for trafficking to the plasma membrane, including GPCRs. Proteins fated for the Golgi apparatus, endoplasmic reticulum, or the nuclear envelope cannot be displayed on VLPs. Efficiency of capture of your protein by VLPs depend on the level of protein expression and the localization of your protein on or near lipid rafts.

Experimental outline

The table below describes the major steps required to synthesize your recombinant membrane protein of interest using the Expi293™ MembranePro™ Expression System. Refer to the specified pages for details to perform each step.

Step	Action	Page
1	Generate the pEF6 expression construct containing your gene of interest	7
2	Culture Expi293F™ Cells	10
3	Co-transfect the Expi293F™ Cells with your pEF6 expression construct and the MembranePro™ Reagent	13
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Methods

Generate the pEF6 expression construct

IMPORTANT! The pEF6/V5-His TOPO® TA Vector Kit (Cat. no. K9610-20) is not included in the Expi293™ MembranePro™ Expression System and must be purchased separately. Visit **www.lifetechnologies.com** or contact support for ordering information.

pEF6/V5-His TOPO® TA Vector Kit

The Expi293™ MembranePro™ Expression System is optimized for use with the pEF6 vector. pEF6 is a non-viral, EF-1α promoter-driven mammalian expression vector that permits the overexpression of your recombinant protein in a broad range of mammalian cell types (Goldman *et al.*, 1996; Mizushima and Nagata, 1990).

The pEF6/V5-His TOPO® TA Vector Kit allows you to directly insert a *Taq* polymerase-amplified PCR product into the pEF6/V5-His TOPO® vector in a highly efficient, 5 minute, one-step cloning ("TOPO® Cloning") reaction to generate your expression vector.

Workflow for generating the pEF6 expression construct

To clone your gene of interest into the pEF6/V5-His-TOPO® vector, perform the steps outlined below and follow the guidelines listed on the next page. For detailed instructions on performing these steps, refer to the pEF6/V5-His TOPO® TA Vector Kit User Guide. This manual is also available for downloading at **www.lifetechnologies.com**.

1. Generate a PCR product containing your gene of interest with a *Taq* DNA polymerase (e.g., Platinum® *Taq* DNA Polymerase).
2. TOPO® Clone your PCR product containing single 3′ A-overhangs into the pEF6/V5-His-TOPO® vector, and use the reaction to transform One Shot® TOP10 Chemically Competent *E. coli*.
3. Pick colonies, isolate plasmid DNA, and screen for insert directionality by sequencing expression clones with primers provided in the kit.

Polymerase mixtures

You may use a polymerase mixture containing *Taq* polymerase and a proofreading polymerase to produce your PCR product; however, the mixture must contain a ratio of *Taq* polymerase:proofreading polymerase in excess of 10:1 to ensure the presence of 3′ A-overhangs on the PCR product.

If you use polymerase mixtures that do not have enough *Taq* polymerase or a proofreading polymerase only, you may add 3′ A-overhangs to your PCR product post-amplification. For more information, refer to the pEF6/V5-His TOPO® TA Vector Kit manual.

Guidelines for generating the expression construct

The cloning of a PCR product into a pEF6/V5-His-TOPO® vector is a rapid and efficient process. However, to ensure proper expression and packaging of your recombinant membrane protein, it is important to pay attention to the general considerations outlined below:

- When using the pEF6/V5-His-TOPO® vector, your insert must contain an ATG initiation codon in the context of a Kozak translation initiation sequence for proper initiation of translation in mammalian cells (Kozak, 1987; Kozak, 1990; Kozak, 1991). An example of a Kozak consensus sequence is provided below. Other sequences are possible, but the G or A at position –3 and the G at position 4 (shown in bold) illustrates the most commonly occurring sequence with strong consensus. Replacing one of the two bases at these positions provides moderate consensus, while having neither results in weak consensus. The ATG initiation codon is shown underlined.

  (G/A)NNATG**G**

- The pEF6/V5-His-TOPO® vector contains the V5 epitope and the C-terminal polyhistidine (6×His) tag (see **Note** below). To express and display a native membrane protein, your insert must contain a stop codon. For this, design your reverse PCR primer to include a stop codon.
- Do not add 5´ phosphates to your primers for PCR. The PCR product synthesized will not ligate into pEF6/V5-His-TOPO® vector.

For detailed instructions for cloning your PCR insert and generating a pEF6 expression construct containing your gene of interest, refer to the pEF6/V5-His TOPO® TA Vector Kit User Guide. It is available for downloading at **www.lifetechnologies.com**.

Note: Cloning your gene into the pEF6/V5-His-TOPO® vector without a stop codon and in frame with the polylinker will result in a fusion protein with V5 and polyhistidine (6×His) tags on the C-terminus of your protein. As the C-terminus of your transmembrane protein will likely be inside the VLP, these tags will be inaccessible to purification resins and antibodies. In theory, these tags could be used to identify and isolate a fusion membrane protein after denaturing the VLP; however, the Expi293™ MembranePro™ Expression System does not support using the tags for extraction and purification.

Guidelines for isolating plasmid DNA

- Plasmid DNA for transfection into eukaryotic cells must be very clean and free from contamination with phenol and sodium chloride. Contaminants may kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency.
- When isolating plasmid DNA from *E. coli* strains (such as TOP10) that are wild type for endonuclease 1 (*end*A1+) with commercially available kits, ensure that the Lysis or Resuspension Buffer contains 10 mM EDTA. EDTA inactivates the endonuclease and avoids DNA nicking and vector degradation.
- Resuspend the purified plasmid DNA in sterile water or TE Buffer, pH 8.0 to a final concentration ranging from 0.1–3.0 µg/mL. You will need 9 µg of the expression plasmid for each transfection.
- To ensure that the plasmid DNA used for transfection is sterile, you may filter-sterilize it through a 0.22 µm filter before use.

IMPORTANT! Do not use mini-prep plasmid DNA for Expi293F™ Cells transfection. We recommend preparing pEF6 plasmid DNA using the PureLink® HiPure Plasmid MaxiPrep kit which contains 10 mM EDTA in the Resuspension Buffer (see page 24 for ordering information).

Guidelines for Culturing Expi293F™ Cells

IMPORTANT! Expi293F™ Cells are not included in the Expi293™ MembranePro™ Expression System. These cells are required, and must be purchased separately. See page 24 for ordering information.

General cell handling

Follow the general guidelines below to grow and maintain Expi293F™ Cells.

- **All solutions and equipment that come in contact with the cells must be sterile.** Always use proper sterile technique and work in a laminar flow hood.
- Before starting experiments, be sure to have cells established and also have some frozen stocks on hand. We recommend using early-passage cells for your experiments. Upon receipt, grow and freeze multiple vials of the Expi293F™ cell line to ensure that you have an adequate supply of early-passage cells.

  Note: To save time and labor, Expi293F™ Cells are also available in convenient 6-vial "Cell Bank" packs to eliminate the need to prepare frozen stocks and verify your own cell bank. See page 24 for ordering information.
- For general maintenance of cells, pass Expi293F™ Cells when they reach a density of approximately 3×10^6–5×10^6 viable cells/mL, typically every 3–4 days.
- Use a hemocytometer with the trypan blue exclusion method or an automated cell counter to determine cell viability. Log phase cultures should be >95% viable.
- When thawing or subculturing cells, transfer cells into pre-warmed medium.

Prepare media

- Expi293™ Expression Medium is formulated with GlutaMAX™-I reagent; for suspension growth and transfection applications, use the Expi293™ Expression Medium without any supplementation.
- Antibiotics are **not** recommended; however, 5 mL/L of Antibiotic-Antimycotic containing penicillin, streptomycin, and amphotericin B may be used when required.

IMPORTANT! Expi293™ Expression Medium is extremely sensitive to light. For optimal results, use and store media protected from light.

Determine cell density and viability

Follow the procedure below to determine viable cell density and percent viability.

1. Aseptically remove a small aliquot of the cell suspension from the culture flask.
2. Determine percent viability and viable cell density using a hemocytometer with the trypan blue exclusion method or an automated cell counter.

Thaw and establish Expi293F™ Cells

Introduction

Follow the protocol below to thaw Expi293F™ Cells to initiate cell culture. Thaw Expi293F™ Cells directly into the Expi293™ Expression Medium supplied with the kit.

Required materials

- Expi293F™ Cells (store frozen cells in liquid nitrogen until ready to use)
- Expi293™ Expression Medium, pre-warmed to 37°C

  Note: We do not recommend adding antibiotics to media as this may negatively impact cell growth.
- 125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask
- Orbital shaker in 37°C incubator with a humidified atmosphere of 8% CO_2
- Room temperature table-top centrifuge and sterile centrifuge tubes
- Reagents and equipment to determine viable and total cell counts (e.g., hemocytometer or an automated cell counter, trypan blue)
- Sterile, 50-mL conical tubes

Thaw Expi293F™ Cells

Store frozen cells in liquid nitrogen until ready to use. To thaw and establish cells:

1. Remove the vial of cells from liquid nitrogen and place it in a 37°C water bath for 1 to 2 minutes to thaw the cells rapidly with gentle agitation. Do **not** submerge the vial in the water.
2. Just before the cells are completely thawed, decontaminate the vial by wiping it with 70% ethanol before opening it in a Class II biological safety cabinet
3. Using a 2-mL or 5-mL pipette, transfer the entire contents of the cryovial into a 125-mL polycarbonate, disposable, sterile, vent-cap Erlenmeyer shaker flask containing 29 mL of pre-warmed Expi293™ Expression Medium.
4. Immediately post-thaw, monitor viable cell density and viability using a hemocytometer with the trypan blue exclusion method or using an automated cell counter. Cell density should be approximately 0.3×10^6 cells/mL and cell viability should be above 80%.
5. Incubate the cells in a 37°C incubator with humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 125 rpm.
6. The day after thawing, determine the viable and total cell counts (see protocol on page 10). Cells viability will drop slightly but should remain above 70% and reach over 90% by day 2–4 post-thaw. Thaw out a new vial of Expi293F™ Cells if the cell viability is below 70%.
7. Once the culture has reached $>1 \times 10^6$ viable cells/mL (~2–4 days), subculture the Expi293F™ Cells by seeding shaker flasks at 3×10^5 viable cells/mL in pre-warmed Expi293™ Expression Medium. We generally use a 125-mL or 250-mL polycarbonate, disposable, sterile, Erlenmeyer flasks containing 30 mL or 60 mL total working volume of cell suspension, respectively.
8. Continue to monitor cell density and viability. Once the culture has reached $>3 \times 10^6$ viable cells/mL (typically 3–4 days), subculture the Expi293F™ Cells by seeding shaker flasks at 3×10^5 viable cells/mL in pre-warmed Expi293™ Expression Medium.

IMPORTANT! Subculture the Expi293F™ Cells a minimum of two additional times to allow them to recover from thawing before using them for transfections.

Subculture Expi293F™ Cells

Guidelines for Passaging Expi293F™ Cells

- Subculture Expi293F™ Cells when they reach a density of approximately 3×10^6–5×10^6 viable cells/mL, typically every 3–4 days.
- Split the Expi293F™ culture to 0.3×10^6–0.5×10^6 cells.
- When maintaining Expi293F™ Cells, we generally use 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 without baffles may be used, but thorough cleaning after each use is essential to avoid potential toxicity, which is more problematic in serum-free cultures.

Passage Expi293F™ Cells

1. Determine viable and total cell counts (see protocol on page 10).
2. Using the cell density determined in Step 1, calculate the split ratio needed to seed the new shaker flask at 0.3×10^6–0.5×10^6 viable cells/mL.
3. Dilute the cells in fresh, pre-warmed Expi293™ Expression Medium to give a final cell density of 0.3×10^6–0.5×10^6 viable cells/mL in the desired final volume.
4. Incubate flasks in a 37°C incubator containing a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 125 rpm.
5. Repeat Steps 1–4 as necessary to maintain or expand cells.

Scale up cell culture

It is possible to scale up the Expi293F™ cultures in spinner flasks or bioreactors. The appropriate spinner or impeller speed and seeding density should be determined and optimized for each system. At Life Technologies, we have determined the optimum spinner speed to be 125 rpm, and impeller speed in Celligen® stirred tank bioreactors to be 70–100 rpm. We recommend seeding the cells at 0.3×10^6–0.5×10^6 viable cells/mL.

Note: If the split ratio of cells to fresh media is less than 1:2, centrifuge the cell suspension and resuspend the cell pellet in fresh, pre-warmed Expi293™ Expression Medium prior to inoculating the spinner or bioreactor culture. Monitor cell viability and the degree of cell clumping. Note that extensive cell clumping may reduce transfection efficiency.

Transfect Expi293F™ Cells

Introduction

To transfect high-density suspension Expi293F™ Cells, you will use the cationic lipid-based transfection reagent, ExpiFectamine™ 293 Reagent, included with the kit. Unlike some other serum-free media formulations, Expi293™ Expression Medium does not inhibit cationic lipid-mediated transfection. Expi293™ Expression Medium is specifically formulated to allow high-efficiency transfection of high-density suspension Expi293F™ Cells **without** the need to change or add media. Transient transfection may be performed in a large volume, allowing larger-scale protein production.

Required materials

- Suspension Expi293F™ Cells cultured in Expi293™ Expression Medium

  Recommendation: Calculate the number of cells that you will need for your transfection experiment and expand cells accordingly. Make sure that the cells are healthy and greater than 95% viable before proceeding to transfection.
- Purified plasmid DNA of interest
- ExpiFectamine™ 293 Reagent (store at 2°C to 8°C until use)
- Opti-MEM® I Reduced-Serum Medium, pre-warmed to 37°C
- Expi293™ Expression Medium, pre-warmed to 37°C

  Note: Do **not** add antibiotics to media during transfection because it may decrease transfection efficiency.
- 125-mL polycarbonate, disposable, sterile Erlenmeyer flasks
- Orbital shaker in a 37°C incubator with a humidified atmosphere of 8% CO_2
- Room temperature table-top centrifuge and sterile, conical centrifuge tubes
- Reagents and equipment to determine viable and total cell counts (e.g., hemocytometer or an automated cell counter, trypan blue)
- Sterile, disposable, polycarbonate snap-cap tubes
- Vortex mixer

Prepare plasmid

- Plasmid DNA for transfection into eukaryotic cells must be clean, sterile, and free from phenol and sodium chloride. Contaminants may kill the cells, and salt will interfere with complexing, decreasing the transfection efficiency. We recommend isolating plasmid DNA using one of the Purelink® HiPure Plasmid Kits (see page 24 for ordering information).

  Note: To ensure sterility, you may filter your DNA preparation through a 0.22 µm-filter before use.
- It is important to accurately quantitate your plasmid DNA stock to prepare optimal DNA:transfection reagent complexes.

Optimal conditions for 36 mL transfection

We generally perform transfections in a final transfection volume of 36 mL; however, transfections can be readily scaled up or down (see **Scale transfections**, page 16). To transfect high-density suspension Expi293F™ Cells, we recommend using the following optimized conditions:

- **Final transfection volume:** 36 mL
- **Number of cells to transfect:** 7.5×10^7 cells with >95% viability
- **Amount of plasmid DNA:** 9 µg
- **Amount of MembranePro Reagent:** 27 µg
- **Amount of ExpiFectamine™ 293 Reagent:** 180 µL.

Transfect Expi293F™ Cells

Follow the procedure below to transfect suspension Expi293F™ Cells in a 36 mL volume. You may keep the cells in Expi293™ Expression Medium during transfection. We recommend including a positive control (Antibody Expressing Positive Control Vector) and a negative control (no DNA, no ExpiFectamine™ 293 Reagent) in your experiment.

1. The day before transfection (day –1), determine the number of cells that you will need for your experiment. For each 36-mL transfection, you will need 7.5×10^7 cells in 26 mL of Expi293™ Expression Medium.
2. The day before transfection (day –1), seed the cells at a density of 2.0×10^6 viable cells/mL and incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker rotating at 125 rpm.
3. On the day of transfection (day 0), determine cell viability using an automated cell counter or the trypan blue dye exclusion method.

   IMPORTANT: Cell density should be $>3 \times 10^6$ cells/mL and the viability of cells must be over 95% to proceed with transfection. Cells should not be clumping.
4. Calculate the volume of cell suspension containing the number of cells needed for one transfection. For each 36 mL (final volume) transfection, you will need 7.5×10^7 cells.
5. Add the appropriate volume of cell suspension to each sterile, disposable 125-mL Erlenmeyer shaker flask and bring up the volume to 26 mL by adding fresh, pre-warmed Expi293™ Expression Medium for each 36 mL (final volume) transfection.
6. Incubate the cells in a 37°C incubator with a humidified atmosphere of 8% CO_2 in air on an orbital shaker rotating at 125 rpm.
7. For each transfection sample, prepare lipid-DNA complexes by performing the following:
   a) Combine 9 µg of plasmid expressing membrane protein of interest with 27 µg of MembranePro™ Reagent, for a total of 36 µg of DNA. Add to Opti-MEM® I Reduced-Serum Medium to a total volume of 4 mL. Mix gently by pipetting up and down 4 times.
   b) Gently mix the ExpiFectamine™ 293 Reagent by pipetting it up and down in a separate tube before use.

Transfect Expi293F™ Cells

c) Dilute 180 uL of ExpiFectamine™ 293 Reagent in 4 mL of Opti-MEM® I Reduced-Serum Medium. Mix gently by pipetting up and down 4 times and incubate for 5 minutes at room temperature.

d) **Note:** Longer incubation times may result in decreased activity.After 5 minute incubation, add the diluted DNA- MembranePro™ Reagent mix to the diluted ExpiFectamine™ 293 Reagent to obtain a total volume of 8 mL. Mix gently by pipetting up and down 4 times.

e) Incubate the mixture for 20 minutes at room temperature to allow DNA-MembranePro™ Reagent-ExpiFectamine™ 293 Reagent complexes to form.

8. After the DNA-MembranePro™ Reagent-ExpiFectamine™ 293 Reagent complex incubation is complete, add 8 mL of DNA-MembranePro™ Reagent-ExpiFectamine™ 293 Reagent complex to each shaker flask from Step 6.

   To the negative control flask, add 8 mL of Opti-MEM® I medium instead of the DNA-ExpiFectamine™ 293 Reagent complex.

   Each flask should now contain a total volume of 34 mL.

9. Incubate the cells in a 37°C incubator with a humidified atmosphere of 8% CO_2 in air on an orbital shaker rotating at 125 rpm.

10. Approximately 18–24 hours post-transfection, add 150 µL of ExpiFectamine™ 293 Transfection Enhancer 1 and 1.5 mL of ExpiFectamine™ 293 Transfection Enhancer 2 to each flask. The final volume should be approximately 36 mL in each 125-mL flask.

    Note: For convenience in adding Enhancers to multiple flasks, you may prepare a cocktail of Enhancer 1 and 2.

11. Cells or media (if recombinant protein is secreted) may be harvested beginning at approximately 48 hours post-transfection and assayed for recombinant protein expression. Time for optimal protein expression depends on the nature of your recombinant protein.

Scale transfections

It is possible to perform transfections in larger or smaller volumes. If you wish to transfect suspension Expi293F™ Cells in a larger or a smaller volume, scale up or down the volume of each reagent accordingly. The table below lists suggested conditions to use when transfecting Expi293F™ Cells at various scales. The optimized conditions to use when transfecting Expi293F™ Cells in a 36 mL volume are listed as a reference. Note that transfection conditions may vary depending on the type of culture vessel used and the growth conditions of your cells; therefore, we recommend performing pilot studies to optimize your transfection conditions.

	Transfection volume				
	1 mL (24-well plate)	2 mL (6-well plate)	36 mL	1 L	3.8 L
Total number of cells	2.1×10^6	4.2×10^6	7.5×10^7	2.1×10^9	8×10^9
Volume of cells	0.72 mL	1.45 mL	26 mL	722 mL	2743 mL
Amount of DNA (plasmid)	0.25 µg	0.5 µg	9 µg	250 µg	950 µg
Amount of MembranePro™ Reagent	0.75 µg	1.5 µg	27 µg	749 µg	1500 µg
DNA dilution volume (in Opti-MEM® I)	to 133 µL	to 222 µL	to 4 mL	to 111 mL	to 422 mL
Amount of ExpiFectamine™ 293 Reagent	5 µL	10 µL	180 µL	5.0 mL	19 mL
ExpiFectamine™ 293 Reagent dilution volume (in Opti-MEM® I)	to 133 µL	to 222 µL	to 4 mL	to 111 mL	to 422 mL
Lipid/DNA complex volume	266 µL	444 µL	8 mL	222 mL	844 mL

Note: The transfection efficiency may decrease as the volume increases if the Expi293F™ Cells are not growing as a single-cell suspension (i.e., if significant cell clumping is observed).

Harvest Virus-Like Particles (VLPs)

Guidelines for harvesting VLPs

- When harvesting the VLPs from the culture medium 48 hours after the transfection, there will likely be floating cell debris. Centrifuge the medium briefly to remove the cell debris. After centrifugation, however, do not collect the last 2 mL of the medium to avoid transferring the pelleted debris.
- You may store the clarified culture medium overnight before isolating the VLPs with the MembranePro™ Precipitation Mix.
- The MembranePro™ Precipitation Mix is slightly viscous. To ensure adequate mixing of the MembranePro™ Precipitation Mix and the culture medium in the collection tube, invert the tube gently at least 10 times. Do **not** vortex.
- Although unnecessary, culture medium containing VLPs may be filtered through a 0.45-micron filter to ensure removal of cells. However, we do not recommend filtration, because it reduces the VLP yield.
- When resuspending the precipitated VLP particles, pipet the solution up and down, taking care not to introduce air bubbles. Do **not** vortex the solution, because it might denature and inactivate your membrane protein of interest.
- You may store the VLPs for 2 days at 4°C, or for up to 6 months at –80°C without any loss of protein activity. Before freezing, aliquot the VLPs to avoid freezing and thawing the particles more than once. Samples which have been subjected to multiple freeze/thaw cycles will exhibit reduced activity.

Materials needed

- Expi293F™ Cells, 48 hour post-transfection
- MembranePro™ Precipitation Mix
- 50-mL conical centrifuge tube
- Phosphate buffered saline (PBS)
- Refrigerated swinging bucket centrifuge (e.g., Sorvall RC3B centrifuge with a HBB-6 rotor)

Harvesting procedure

1. Collect the growth medium containing the VLPs from the culture flask by decanting, and transfer it to a 50-mL conical centrifuge tube.
2. Centrifuge the medium containing the VLPs at 3,000 – 5,000 × g for 15 minutes in a centrifuge with a swinging bucket rotor to pellet and remove the cell debris.
3. Transfer culture supernatant to a fresh tube and repeat centrifugation to remove any contaminating cells or debris. This double centrifugation step is essential due to the high cell density of Expi293F™ cultures.
4. Carefully transfer clarified sample to a fresh 50-mL conical centrifuge tube using a pipette. Do **not** attempt to transfer the last 2 mL of the sample to avoid transferring any pelleted material.
5. Add 1/5 volume of MembranePro™ Precipitation Mix to the clarified medium (i.e., if harvested medium is 25 mL, add 5 mL of MembranePro™ Precipitation Mix).
6. Mix the sample gently by inverting the tube 10 times until the MembranePro™ Precipitation Mix is entirely incorporated into the medium.
7. Incubate the sample at 4°C overnight (at least 18 hours).
8. Recover the VLP particles by centrifuging the sample at 3,000 – 5,000 × g for 15 minutes in a refrigerated swinging bucket centrifuge.
9. Carefully remove the medium by decanting or pipetting. Make sure not to dislodge the VLP pellet.

   Note: Pelleted particles may or may not be clearly visible following centrifugation.
10. Resuspend particles in 1x PBS for use or aliquot for storage at –80°C.
11. If complete removal of the culture medium is required for downstream applications (e.g., for protein determination on VLPs), rinse the centrifuge tube with MembranePro™ Precipitation Mix diluted 1:5 in 1X PBS. Otherwise, proceed to step 14.
12. Centrifuge the sample again for 5 minutes at 3,000 – 5,000 × g for 5 minutes in a swinging bucket centrifuge.
13. Carefully remove the supernatant by pipetting.
14. Resuspend the VLP pellet in 500 µL of PBS or the desired amount of assay buffer by repeatedly pipetting it up and down. Be careful not to create froth in the sample. Do **not** vortex. The resuspended sample will appear slightly turbid.

    Note: Any particulate matter that cannot be resuspended by repeated pipetting or by smearing against the tube wall using the pipettor tip can be left in suspension or separated from the sample by allowing it to settle to the bottom of the tube.
15. Proceed to the desired assay. You may also store the VLPs at 4°C for 2 days or at –80°C for up to 6 months without any loss of activity if the samples are not subjected to repeated freeze/thaw cycles.

Troubleshooting

Generating the pEF6 Expression Construct

For troubleshooting the problems you may encounter while generating your pEF6 expression construct containing your gene of interest, refer to the pEF6/V5-His TOPO® TA Vector Kit User Guide.

Transfection

The table below lists some potential problems and solutions that help you troubleshoot the transfection step in your membrane protein expression experiments.

Symptom	Cause	Solution
No VLPs recovered and the control transfection did not work	Expi293F™ Cells are unhealthy	Ensure that the cells are healthy and greater than 90% viable before transfection.
		Use cells that have been subcultured for less than 16 passages.
		Use cells that have been passaged 3–4 times after the most recent thaw.
	Culture density of Expi293F™ Cells is too high	Do not allow cells to overgrow.
		Check cell density before transfection and adjust as necessary.
	Transfection unsuccessful	Follow the transfection protocol exactly as described in protocol. During transfection, pay particular attention to the following points: • Do **not** include antibiotics in the medium. • Use Opti-MEM® I Reduced-Serum Medium to dilute transfection complexes. • Refer to Expi293F™ Cells User Guide
	Used cells other than Expi293F™ Cells	• If you are using a cell line other than Expi293F™ Cells (i.e., a stable cell line), we recommend testing your cell line with the pEF6/V5-His-TOPO®/*lacZ* control (or a similar reporter construct) to determine its transfectability with ExpiFectamine™ 293 Reagent before attempting VLP formation.

MembranePro™ Procedures

The table below lists some potential problems and solutions that help you troubleshoot the expression and VLP harvest steps in your membrane protein expression experiments.

Symptom	Cause	Solution
No or little VLPs recovered, but the control transfection worked	pEF6 expression construct is not pure	Plasmid DNA for transfection into eukaryotic cells must be very clean and free from contamination with phenol and sodium chloride. Isolate the expression construct using the PureLink® HiPure Plasmid MaxiPrep kit.
	Ratio of expression construct to MembranePro™ Reagent is incorrect	Co-transform Expi293F™ Cells with 9 µg of purified pEF6 expression construct and 27 µg of MembranePro™ Reagent to maintain a 1:3 ratio.
	MembranePro™ Precipitation Mix is not mixed well enough	The MembranePro™ Precipitation Mix is slightly viscous. To ensure adequate mixing of the MembranePro™ Precipitation Mix and the culture medium in the collection tube, invert the tube gently at least 10 times. Do **not** vortex.
	Cells incubated for too long after the transfections	If transfections are performed too early in the afternoon, VLPs may be secreted into the growth medium containing the transfection complexes during the night. These VLPs will be lost when the medium in changed in the morning.
Serum proteins appear in the VLP precipitate	Used non-recommended sera in the media	Prepare media using the recommended fetal bovine serum (FBS).
Membrane protein is not functional or shows reduced activity	VLPs damaged during harvest	When resuspending the precipitated VLP particles, pipet the solution up and down, taking care not to introduce air bubbles. Do **not** vortex the solution, because it might denature and inactivate your membrane protein of interest.
	VLPs stored incorrectly before the functional assay	You may store the VLPs for 2 days at 4°C, or for up to 6 months at –80°C without any loss of protein activity.
		Before storing, aliquot the VLPs to avoid freezing and thawing the particles more than once. Samples which have been subjected to multiple freeze/thaw cycles will exhibit reduced activity.
	Protein of interest is not a membrane protein associated with the plasma membrane lipid rafts	You can use the Expi293™ MembranePro™ Expression System to display most membrane proteins that are destined for the plasma membrane, including GPCRs. Proteins fated for the Golgi apparatus, endoplasmic reticulum, or the nuclear envelope cannot be displayed on VLPs.

Symptom	Cause	Solution
Membrane protein is not functional or shows reduced activity	VLPs damaged during harvest	When resuspending the precipitated VLP particles, pipet the solution up and down, taking care not to introduce air bubbles. Do **not** vortex the solution, because it might denature and inactivate your membrane protein of interest.
	VLPs stored incorrectly before the functional assay	You may store the VLPs for 2 days at 4°C, or for up to 6 months at –80°C without any loss of protein activity.
		Before storing, aliquot the VLPs to avoid freezing and thawing the particles more than once. Samples which have been subjected to multiple freeze/thaw cycles will exhibit reduced activity.
	Protein of interest is not a membrane protein associated with the plasma membrane lipid rafts	You can use the Expi293™ MembranePro™ Expression System to display most membrane proteins that are destined for the plasma membrane, including GPCRs. Proteins fated for the Golgi apparatus, endoplasmic reticulum, or the nuclear envelope cannot be displayed on VLPs.

Appendix A

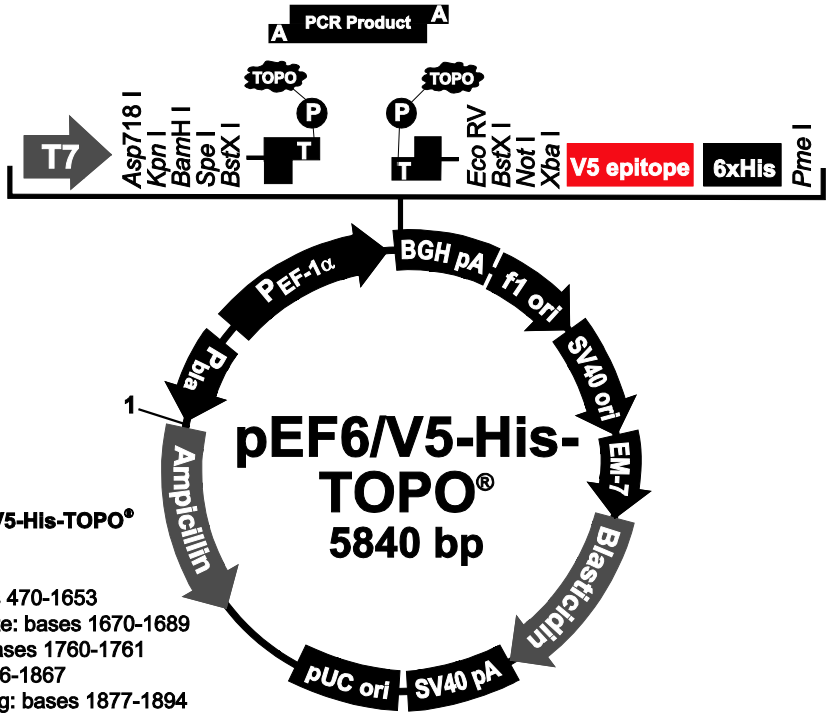
pEF6/V5-His-TOPO® Vector

Map of pEF6/V5-His-TOPO® vector

The figure below summarizes the features of the pEF6/V5-His-TOPO® vector. The vector is supplied linearized between base pairs 1,760 and 1,761. This is the TOPO® Cloning site. Unique restriction sites flanking the TOPO® Cloning site are shown.

Note: The vector kit must be purchased separately.

For more information on the pEF6/V5-His-TOPO® Vector, refer to the pEF6/V5-His TOPO® TA Vector Kit User Guide. The complete sequence for pEF6/V5-His-TOPO® is available for downloading at **www.lifetechnologies.com** or by contacting Technical Support (see page 25).



Comments for pEF6/V5-His-TOPO®
5840 nucleotides

EF-1α promoter: bases 470-1653
T7 promoter/priming site: bases 1670-1689
TOPO® Cloning site: bases 1760-1761
V5 epitope: bases: 1826-1867
Polyhistidine (6xHis) tag: bases 1877-1894
BGH reverse priming site: bases 1917-1934
BGH polyadenylation signal: bases 1923-2147
f1 origin of replication: bases 2193-2621
SV40 promoter and origin: bases 2626-2970
EM-7 promoter: bases 3012-3078
Blasticidin resistance gene: bases 3079-3477
SV40 early polyadenylation signal: bases 3635-3765
pUC origin: bases 4148-4821 (complementary strand)
bla promoter: bases 21-105 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 4966-5826 (complementary strand)

Features of pEF6/V5-His-TOPO® Vector

pEF6/V5-His-TOPO® Vector(5,840 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
Human elongation factor 1α (hEF-1α) promoter	Permits overexpression of your recombinant protein in a broad range of mammalian cell types (Goldman *et al.*, 1996; Mizushima and Nagata, 1990)
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert
TOPO® Cloning site	Allows insertion of your PCR product in frame with the C-terminal V5 epitope and polyhistidine (6×His) tag
V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr)	Allow detection and purification of the fusion protein; however, fusion displayed on VLPs normally contain the V5 epitope and the polyhistidine (6×His) tag inside the VLP, which is inaccessible to purification resins and antibodies. In theory, these tags could be used to identify and isolate a fusion membrane protein after denaturing the VLP; however, the MembranePro™ Functional Protein Expression System does not support using the tags for extraction and purification.
C-terminal polyhistidine (6×His) tag	
BGH reverse priming site	Permits sequencing through the insert
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
f1 origin	Allows rescue of single-stranded DNA
SV40 early promoter and origin	Allows efficient, high-level expression of the blasticidin resistance gene and episomal replication in cells expressing the SV40 large T antigen
EM-7 promoter	For expression of the blasticidin resistance gene in *E coli*
Blasticidin resistance gene (*bsd*)	Selection of stable transfectants in mammalian cells (Kimura *et al.*, 1994)
SV40 polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA
pUC origin	High-copy number replication and growth in *E. coli*
bla promoter	Allows expression of the ampicillin (*bla*) resistance gene
Ampicillin resistance gene (β-lactamase)	Selection of transformants in *E. coli*

Accessory products

Required materials not included in the kit

The pEF6 V5-His TOPO® Expression Kit and Expi293F™ Cells must be purchased separately. For more information, refer **www.lifetechnologies.com** or contact Technical Support (see page 25).

Product	Amount	Cat. no.
pEF6 V5-His TOPO® TA Expression Kit	20 reactions	K9610-20
Expi293F™ Cells	1 mL	A14527
	6 × 1 mL	A14528

Individual kit components

Individual components of the kit can be purchased separately. For more information, refer **www.lifetechnologies.com** or contact Technical Support (see page 25).

Product	Amount	Cat. no.
Expi293™ Expression Medium	1 L	A1435101
	6 × 1 L	A1435102
ExpiFectamine™ 293 Transfection Kit*	1 × 1 L culture	A14524
	1 × 10 L culture	A14525
	5 × 10 L culture	A14526
Opti-MEM® I Reduced Serum Medium	100 mL	31985-062
	500 mL	31985-070

*This kit contains ExpiFectamine™ 293 Reagent, and ExpiFectamine™ 293 Transfection Enhancers 1 and 2.

Additional products

The products listed below may be used with the Expi293™ MembranePro™ Expression System. For more information, refer **www.lifetechnologies.com** or contact Technical Support (see page 25).

Product	Amount	Cat. no.
Platinum® *Taq* DNA Polymerase	100 reactions 500 reactions	10966-018 10966-034
PureLink® HiPure Plasmid MaxiPrep kit	10 preps 25 preps	K2100-06 K2100-07
Trypan Blue Stain	100 mL	15250-061
Water, distilled	20 × 100 mL	15230-196

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com)**
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/sds**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies and/or its affiliate(s) warrant their products as set forth in the Life Technologies General Terms and Conditions of Sale found on the Life Technologies web site at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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