USER GUIDE

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by life technologies™

MembranePro™ Functional Protein Expression System

For capture and display of functional membrane proteins using virus-like particles

Catalog Numbers A11667, A11668, A11669, and A11670

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Contents

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Amount	Cat. no.
MembranePro™ Functional Protein Expression Kit	10 reactions	A11667
MembranePro™ Functional Protein Support Kit	10 reactions	A11668
	60 reactions	A11669
	600 reactions	A11670

Kit Components

Each product contains the following components. For detailed descriptions of the components, see pages 2–4.

Component	Size	Quantity
MembranePro™ Functional Protein Expression Kit – 10 reactions		
MembranePro™ Reagent	300 µg/tube	1 tube
MembranePro™ Precipitation Mix	75 mL/bottle	1 bottle
Lipofectamine® 2000 Transfection Reagent	0.75 mL/tube	3 tubes
293FT Cells	3×10^6 cells/vial	1 vial
pEF6/V5-His TOPO® TA Vector Kit	20 reactions/kit	1 kit
One Shot® TOP10 Chemically Competent *E. coli*	20 reactions/kit	1 kit
MembranePro™ Functional Protein Support Kit – 10 reactions		
MembranePro™ Reagent	300 µg/tube	1 tube
MembranePro™ Precipitation Mix	75 mL/bottle	1 bottle
Lipofectamine® 2000 Transfection Reagent	0.75 mL/tube	3 tubes
MembranePro™ Functional Protein Support Kit – 60 reactions		
MembranePro™ Reagent	300 µg/tube	6 tubes
MembranePro™ Precipitation Mix	75 mL/bottle	5 bottles
Lipofectamine® 2000 Transfection Reagent	15 mL/kit	1 kit
MembranePro™ Functional Protein Support Kit – 600 reactions		
MembranePro™ Reagent	300 µg/tube	10 × 6 tubes
MembranePro™ Precipitation Mix	75 mL/bottle	10 × 5 bottles
Lipofectamine® 2000 Transfection Reagent	15 mL/kit	10 kits

Product Use

The MembranePro™ Functional Protein Expression Kit and all its components are for research use only. They are not intended for any animal or human therapeutic or diagnostic use.

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Kit Contents and Storage, continued

Shipping and Storage

Upon receipt, store each component of the MembranePro™ Functional Protein Expression System as detailed below.

Item	Shipping	Storage
MembranePro™ Precipitation Mix	Room temperature	Room temperature
MembranePro™ Reagent	Wet ice	–20°C
Lipofectamine® 2000	Wet ice	4°C **(do not freeze)**
293FT Cells	Dry ice	Liquid nitrogen
pEF6 V5-His TOPO® TA Vector Kit	Dry ice	–20°C
One Shot® TOP10 Chemically Competent *E. coli*	Dry ice	–80°C

293FT Cells

The 293FT Cell Line is supplied as one vial containing 3×10^6 frozen cells in 1 mL of Freezing Medium. Note that only the MembranePro™ Functional Protein Expression Kit includes the 293FT producer cell line. **Upon receipt, store the cells in liquid nitrogen.**

For additional instructions and information on the 293FT cell line, see the 293FT Cell Line manual, included in the MembranePro™ Functional Protein Expression Kit. The 293FT Cell Line manual is also available at **www.lifetechnologies.com**, or by contacting Technical Support (see page 31).

The MembranePro™ protocol and media recommendations are optimized for use with 293FT cells for the production of MembranePro™ particles and they may deviate slightly from the recommendations made in the 293FT cell manual. For optimal results, follow the media and culture protocol for MembranePro™ particle production specified in this manual.

Lipofectamine® 2000

The Lipofectamine® 2000 reagent supplied with the kit is a proprietary, cationic lipid-based formulation suitable for the transfection of nucleic acids into eukaryotic cells. Lipofectamine® 2000 provides the highest transfection efficiency in 293FT cells. If you are using a cell line other than 293FT (i.e., a stable cell line), we recommend testing your cell line with the *lac*Z control included in the pEF6 V5-His TOPO® TA Vector Kit (or a similar reporter construct) to determine its transfectability with Lipofectamine® 2000. **Upon receipt, store the Lipofectamine® 2000 Reagent at 4°C. Do not freeze.**

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Kit Contents and Storage, continued

pEF6 V5-His TOPO® TA Vector Kit

pEF6/V5-His TOPO® TA Vector Kit, included in the MembranePro™ Functional Protein Expression Kit and also available separately (see page 29), contains the reagents listed below. **Upon receipt, store the vector kit at –20°C.**

Reagent	Composition	Amount
pEF6/V5-His-TOPO® vector	10 ng/µL plasmid DNA	20 µL
10X PCR Buffer	100 mM Tris-HCl, pH 8.3 500 mM KCl 25 mM $MgCl_2$ 0.01% gelatin	100 µL
dNTP Mix	12.5 mM dATP 12.5 mM dCTP 12.5 mM dGTP 12.5 mM dTTP	10 µL
Salt Solution	1.2 M NaCl 0.06 M $MgCl_2$	50 µL
T7 Promoter Primer	0.1 µg/µL in TE Buffer, pH 8.0	20 µL
BGH Reverse Primer	0.1 µg/µL in TE Buffer, pH 8.0	20 µL
Control PCR Template	0.05 µg/µL in TE Buffer, pH 8.0	10 µL
Control PCR Primers	0.1 µg/µL **each** in TE Buffer, pH 8.0	10 µL
Sterile Water	—	1 mL
Expression Control Plasmid (pEF6/V5-His-TOPO®/*lacZ*)	0.5 mg/mL in TE Buffer, pH 8.0	10 µL

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Kit Contents and Storage, continued

One Shot® TOP10 Chemically Competent *E. coli*

One Shot® TOP10 Chemically Competent *E. coli*, included in the MembranePro™ Functional Protein Expression Kit, are supplied with the following reagents. Transformation efficiency of the competent cells is ≥1 × 10^8 cfu/µg plasmid DNA. **Store the TOP10 cells at –80°C.**

Reagent	Composition	Amount
S.O.C. Medium	2% Tryptone 0.5% Yeast Extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 20 mM glucose	6 mL
TOP10 Chemically Competent *E. coli*	—	21 × 50 µL
pUC19 Control DNA	10 pg/µL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8.0	50 µL

Genotype of TOP10 Cells

F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*) 7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G λ$^-$

Description of the System

MembranePro™ Functional Protein Expression

MembranePro™ Functional Protein Expression Technology

The MembranePro™ Functional Protein 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 MembranePro™ Functional Protein Expression Works

Virus-like particles (VLPs) are subviral particles that self-assemble from virus-derived core structural proteins (HIV-1 gag/pol, rev, and VSV-G). Because VLPs lack a viral genome, none of the structural genes are packaged into MembranePro™ particles. As the system does not provide a nucleic acid payload, the resultant particles are non-transducing, non-infectious, and non-replicating.

The MembranePro™ Functional Protein Expression System takes advantage of the functionality of the lentiviral gag protein, which, when expressed in 293FT 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 MembranePro™ 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**

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MembranePro™ Functional Protein Expression, continued

Components of MembranePro™ Functional Protein Expression System

The MembranePro™ Functional Protein Expression System is offered in two configurations: The MembranePro™ Functional Protein Expression Kit, which provides the convenience of a complete kit with all the reagents supplied for 10 reactions, and the MembranePro™ Functional Protein Support Kit, which includes the reagents for functional expression of membrane proteins but does not contain the expression vector cloning module or the 293FT host cells. The MembranePro™ Functional Protein Support Kit is offered in three sizes, allowing 10, 60, or 600 reactions.

- MembranePro™ Reagent optimized for high-yield packaging and secretion of VLPs that contain the functional membrane protein of interest into the extracellular medium
- MembranePro™ Precipitation Mix 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
- Lipofectamine® 2000 Transfection Reagent for simple, high-efficiency transfection of the pEF6 expression construct into the host 293FT cells
- 293FT cells for high-level expression of the recombinant membrane protein as well as the VLP packaging proteins (supplied with the MembranePro™ Functional Protein Expression Kit or available separately; see page 29 for ordering information)
- pEF6 V5-His TOPO® TA Vector Kit for generating the pEF6 expression vector containing your gene of interest in a highly efficient, 5 minute, one-step TOPO® Cloning reaction (supplied with the MembranePro™ Functional Protein Expression Kit or available separately; see page 29 for ordering information)
- One Shot® TOP10 Chemically Competent *E. coli* for selecting and amplifying the pEF6 expression vector containing your gene of interest for subsequent transfection into 293FT cells

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MembranePro™ Functional Protein Expression, continued

Advantages of MembranePro™ Functional Protein 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.
- Allows the production of VLPs using the scalable FreeStyle™ 293 Expression System as an alternative (see page 22).

Possible Applications

VLPs produced using the MembranePro™ Functional Protein Expression Kits 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.

You can use the MembranePro™ Functional Protein 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.

As an alternative to the 293FT cells provided with the MembranePro™ Functional Protein Expression Kit, you can also use the scalable FreeStyle™ 293 Expression System to generate VLPs (see page 22 for the alternative protocol).

Continued on next page

Experimental Outline

The table below describes the major steps required to synthesize your recombinant membrane protein of interest using the MembranePro™ Functional Protein Expression Kit. 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	9–11
2	Co-transfect the 293FT producer cell line with your pEF6 expression construct and the MembranePro™ Reagent	12–14
3	Collect culture medium and clarify it by low speed centrifugation	15–17
4	Harvest VLPs using the MembranePro™ Precipitation Mix	17
5	Resuspend VLPs and proceed to functional assay or aliquot the VLPs and freeze for future use	17

Methods

Generate the pEF6 Expression Construct

pEF6/V5-His TOPO® TA Vector Kit

The MembranePro™ Functional Protein 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).

For your convenience, the pEF6/V5-His TOPO® TA Vector Kit is supplied with the MembranePro™ Functional Protein Expression Kit, and it is also available separately from Life Technologies (see page 29). 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 manual (part no. 25-0279). This manual is supplied as a component of the MembranePro™ Functional Protein Expression Kit, and it 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.

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Generate the pEF6 Expression Construct, continued

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)NN<u>ATG</u>**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 manual (part no. 25-0279). This manual is supplied as a component of the MembranePro™ Functional Protein Expression Kit, and it is also available for downloading at **www.lifetechnologies.com**.

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 MembranePro™ Functional Protein Expression System does not support using the tags for extraction and purification.

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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 293FT 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 30 for ordering information).

Transfect 293FT Cells

CAUTION! When working with mammalian 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**.

293FT Cell Line

The human 293FT Cell Line is supplied with the MembranePro™ Functional Protein Expression Kit to facilitate optimal viral-like particle (VLP) production.

For more information on culturing and maintaining 293FT cells, refer to the 293FT Cell Line manual (part no. 25-0504). This manual, supplied with the MembranePro™ Functional Protein Expression Kit, is also available at **www.lifetechnologies.com** or by contacting Technical Support (see page 31).

Note: The 293FT Cell Line is also available separately from Life Technologies (page 29).

The MembranePro™ protocol and media recommendations are optimized for use with 293FT cells for the production of VLPs and they may deviate slightly from the recommendations made in the 293FT cell manual. For optimal results, follow the media and culture protocol for VLP production specified in this manual.

Transfection Methods

The 293FT Cell Line is generally amenable to transfection using standard methods including calcium phosphate precipitation (Chen & Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated transfection (Felgner *et al.*, 1989; Felgner & Ringold, 1989), and electroporation (Chu *et al.*, 1987; Shigekawa & Dower, 1988). We typically use cationic lipid-based transfection reagents to transfect 293FT cells and recommend the Lipofectamine® 2000 transfection reagent for best results.

Lipofectamine® 2000

The Lipofectamine® 2000 reagent supplied with the MembranePro™ kits (Ciccarone *et al.*, 1999) is a proprietary, cationic lipid-based formulation suitable for the transfection of nucleic acids into eukaryotic cells. Using Lipofectamine® 2000 to transfect 293FT cells offers the following advantages:

- Provides the highest transfection efficiency in 293FT cells.
- You can add the DNA-Lipofectamine® 2000 complexes directly to cells in culture medium in the presence of serum.
- You do not have to remove the complexes or change or add medium following transfection; however, you may remove the complexes 4–6 hours after transfection without loss of activity.

Note: Lipofectamine® 2000 is also available separately from Life Technologies (see page 29).

Opti-MEM® I

To facilitate optimal formation of DNA-Lipofectamine® 2000 complexes, we recommend using Opti-MEM® I Reduced Serum Medium (see page 29).

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Transfect 293FT Cells, continued

Guidelines for Transfection

The health of your 293FT cells at the time of transfection has a critical effect on the success of VLP production. Use of "unhealthy" cells will negatively affect the transfection efficiency, resulting in decreased amount of VLP production. For optimal VLP production, follow the guidelines below to culture 293FT cells before their use in transfection:

- Ensure that the cells are healthy and greater than 90% viable.
- Ensure that the monolayer is 90% confluent at time of transfection to avoid cytotoxicity, detachment of cells during manipulation, and optimal VLP yield.
- Subculture and maintain cells in complete D-MEM medium containing 4% Fetal Bovine Serum (FBS), 0.1 mM MEM Non-Essential Amino Acids, 1 mM sodium pyruvate, and 4 mM L-glutamine.
  Note: Using sera other than those recommended in this manual may result in serum proteins co-precipitating with VLPs in the presence of MembranePro™ Precipitation mix.
- Discontinue using antibiotics at least one passage before transfection.
- Do not allow cells to grow to confluency.
- Use cells that have been passaged 3–4 times after the most recent thaw.
- Use cells that have been subcultured for less than 16 passages.
- Since 293FT cells are easily dislodged, do not squirt transfection complexes directly onto cell monolayer.

If transfections are performed too early in the afternoon, protein production and VLP formation may begin during the night resulting in particle secretion into the growth medium containing the transfection complexes. These VLPs will be lost when the medium in changed in the morning.

For convenience and to maximize particle yield, we recommend performing the transfections in the late afternoon (after 4 pm) with a medium change the following morning (before 10 am).

Materials Needed

- MembranePro™ Reagent
- pEF6 expression construct, purified according to guidelines on page 11
- 293FT cells, at 90% confluency (see next page)
- D-MEM complete growth medium supplemented with 0.1 mM MEM Non-Essential Amino Acids, 1 mM sodium pyruvate, and 4 mM L-glutamine, pre-warmed to 37°C
- Lipofectamine® 2000 transfection reagent, mix gently before use
- Opti-MEM® I Reduced Serum Medium
- T-175 culture flasks
- 15-mL conical tubes
- 37°C incubator with a humidified atmosphere of 5% CO_2

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Transfect 293FT Cells, continued

Transfection Procedure

Day 1 (Preparing 293FT Cells for Transfection):

1. The day before transfection, plate approximately 1×10^7 293FT cells in 25 mL of complete growth medium in a T-175 tissue culture flask (see previous page) for each transfection. **Do not include antibiotics in the medium.**

   Note: If plating a stable cell line other than 293FT for VLP production, ensure that the cells are 90% confluent on the day of transfection and that they can be transfected with at least 70% efficiency.

2. Incubate cells overnight in a 37°C incubator with a humidified atmosphere of 5% CO_2. Make sure that the cells are 90% confluent on the day of transfection.

Day 2 (Transfection):

3. Prepare transfection complexes **for each transfection** as follows:

   a. In a sterile 15-mL tube, combine 9 µg of purified pEF6 expression construct, 27 µg of MembranePro™ Reagent, and 4 mL of Opti-MEM® I Reduced Serum Medium. Mix gently.

      Note: If you are using a stable cell line other than 293FT, combine 36 µg of MembranePro™ Reagent and 4 mL of Opti-MEM® I Reduced Serum Medium in a sterile 15-mL tube, and mix gently.

   b. In a separate sterile 15 mL tube, combine 180 µL of Lipofectamine® 2000 (mix gently before use) and 4 mL of Opti-MEM® I Reduced Serum Medium.

   Mix the tubes gently and incubate for 5 minutes at room temperature.

   Note: Proceed to Step 4 within 25 minutes.

4. After incubation, combine the diluted DNA (Step a) with the diluted Lipofectamine® 2000 (Step b). Mix gently.
5. Incubate the mixture for 20 minutes at room temperature to allow the DNA-Lipofectamine® 2000 complexes to form. The solution may appear cloudy, but this will not impede the transfection.

   Note: The complexes are stable for 6 hours at room temperature.

6. Add the DNA-Lipofectamine® 2000 complexes (Step 5) **dropwise** to the T-175 tissue culture flask containing the 293FT cells in 25 mL of complete growth medium. Mix gently by rocking the plate back and forth, taking care not to dislodge the cells.
7. Incubate the cells overnight at 37°C in a humidified 5% CO_2 incubator (for approximately 18 hours after transfection).

Day 3:

8. The next morning (Day 3), remove and discard the medium containing the DNA-Lipofectamine® 2000 complexes, and replace it with 32 mL of complete culture medium **without antibiotics**.
9. Incubate cells for another day at 37°C in a humidified 5% CO_2 incubator. The VLPs begin to bud off from the cell membrane and get secreted into the culture medium approximately 48 hours after transfection.

   Note: During VLP production 293FT cells may fuse, resulting in the appearance of large, multinucleated cells known as syncytia. This morphological change is normal and does not affect production of the VLPs.

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Positive Control

You can use the pEF6/V5-His-TOPO®/*lacZ* expression vector as a positive control for mammalian transfection and expression. The gene encoding β-galactosidase is expressed in mammalian cells under the control of the human EF-1α promoter. A successful transfection will result in β-galactosidase expression that can be easily assayed (see below). However, you cannot use this vector as a positive control for VLP formation and secretion, because β-galactosidase is not a membrane protein and it will not be incorporated into the VLPs budding from the 293FT plasma membrane and secreted into the growth medium.

If you are using a cell line other than 293FT (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 Lipofectamine® 2000 before attempting VLP formation. The *lacZ* control is provided with the pEF6/V5-His TOPO® TA Vector Kit as part of the MembranePro™ Functional Protein Expression Kit. For more information, including the map of the vector and a description of its features, refer to the pEF6/V5-His TOPO® TA Vector Kit manual (part no. 25-0279).

Assay for ß-galactosidase Activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972) or by staining the cells for activity. Life Technologies offers the β-Gal Assay Kit and the β-Gal Staining Kit for fast and easy detection of β-galactosidase expression. See page 30 for ordering information.

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 pelletted 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.
- One T-175 flask yields approximately 500 µL of VLPs following resuspension. Depending on the efficiency of expression of the membrane protein of interest, a single reaction may generate a VLP sample containing 50 µg to 100 µg of total protein.

CAUTION! When working with mammalian 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**.

CAUTION! Although the MembranePro™ 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**.

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Harvest Virus-Like Particles (VLPs), continued

Materials Needed

- 293FT 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 2,000 × *g* for 10 minutes in a clinical centrifuge with a swinging bucket rotor to pellet the cell debris.
3. 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 pelletted material.
4. 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).
5. Mix the sample gently by inverting the tube several times until the MembranePro™ Precipitation Mix is entirely incorporated into the medium.
6. Incubate the sample at 4°C overnight (at least 18 hours).
7. Recover the VLP particles by centrifuging the sample at 5,500 × *g* for 30 minutes in a refrigerated swinging bucket centrifuge.

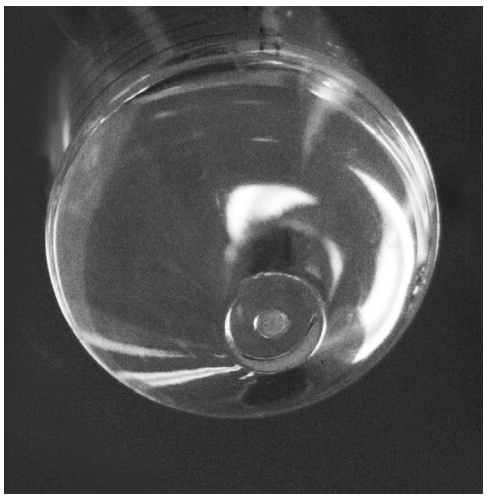
   Note: Following centrifugation, if packaging was successful, a yellow-white disc or pellet of VLPs should be visible at the bottom of the conical tube (see next page).
8. Carefully remove the medium by decanting or pipetting. Make sure not to dislodge the VLP pellet.
9. If complete removal of the culture 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 12.
10. Centrifuge the sample again for 5 minutes at 5,500 × *g* for 5 minutes in a swinging bucket centrifuge.
11. Carefully remove the supernatant by pipetting.
12. 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.
13. 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.

Expected Results

Successful VLP Packaging

If VLP packaging was successful, a yellow-white pellet of VLPs should be visible at the bottom of the conical tube following centrifugation (step 7, previous page). This pellet may not be as distinct as in the pictures below; however, when the VLP pellet is resuspended in PBS (Step 12, previous page), the resulting solution will appear slightly cloudy. Be sure to rinse the walls of the cone during resuspension to retrieve all VLPs.



A **B**

Panels A and B show the images of the 50-mL tube containing the VLPs before and after the centrifugation step (step 7, previous page), respectively. The VLP pellet is clearly visible at the bottom of the tube after centrifugation.

Expected Yield

One T-175 flask yields approximately 500 µL of VLPs following resuspension. VLP protein concentration depends on protein expression and purification efficiency, and may be between 0.1 µg/µL and 1 µg/µL. Depending on the efficiency of expression of your GPCR and the activity of the GPCR for your ligand of interest, a single reaction may generate sufficient VLP sample for up to 1000 ligand binding assay data points.

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 manual (part no. 25-0279).

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	293FT 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.
	Density of 293FT culture is too high	Do not allow cells to grow to confluency.
		Ensure that the monolayer is 90% confluent at time of transfection to avoid cytotoxicity and detachment of cells during manipulation.
	Transfection unsuccessful	Follow the transfection protocol exactly as described on pages 12–15. 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. • Because 293FT cells are easily dislodged, do not squirt transfection complexes directly onto cell monolayer.
	Used cells other than 293FT	If you are using a cell line other than 293FT (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 Lipofectamine® 2000 before attempting VLP formation.

Continued on next page

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 293FT 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. For convenience and to maximize particle yield, we recommend performing the transfections in the late afternoon (after 4 pm) with a medium change the following morning (before 10 am).
Serum proteins appear in the VLP precipitate	Used non-recommended sera in the media	Prepare media using the recommended fetal bovine serum (FBS) (see page 29 for ordering information).

Continued on next page

MembranePro™ Procedures, continued

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 MembranePro™ Functional Protein 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

Scalable Production of Virus-Like Particles (VLPs) in FreeStyle™ 293-F Cells

VLP Production in FreeStyle™ 293-F Cells

You can use the FreeStyle™ 293 Expression System to generate the VLPs in FreeStyle™ 293-F suspension adapted cells (see page 30 for ordering information).

The FreeStyle™ 293-F cell line is a variant of the 293 cell line that has been adapted to high density, serum-free, suspension growth in FreeStyle™ 293 Expression Medium. The FreeStyle™ 293-F cells demonstrate high transfection efficiencies with the 293fectin™ transfection reagent and allow transfections to be performed at large volumes, facilitating larger scale VLP production. In addition, unlike some other serum-free media formulations, FreeStyle™ 293 Expression Medium does not inhibit cationic lipid-mediated transfection and permits high efficiency transfections **without** the need to change or add media.

A brief protocol for transfecting FreeStyle™ 293-F cells is provided below; for more information on maintaining and transfecting FreeStyle™ 293-F cells in suspension culture, refer to the FreeStyle™ 293 Expression System manual (part no. 25-0439), available at **www.lifetechnologies.com** or by contacting Technical Support (see page 31).

Materials Needed

In addition to the MembranePro™ Functional Protein Expression Kit, the following materials are needed for the scalable production of VLPs in FreeStyle™ 293-F cells. See page 30 for ordering information.

- Suspension FreeStyle™ 293-F cells cultured in FreeStyle™ 293 Expression Medium

  Recommendation: Calculate the number of cells that you need for your transfection experiment and expand cells accordingly. Make sure that the cells are healthy and greater than 90% viable before proceeding to transfection.
- MembranePro™ Reagent
- pEF6 expression construct, purified according to guidelines on page 11
- 293fectin™ transfection reagent (store at 4°C until use)
- Opti-MEM® I Reduced Serum Medium, pre-warmed to 37°C
- FreeStyle™ 293 Expression Medium, pre-warmed to 37°C

  Note: Do not add antibiotics to media during transfection, because antibiotics 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 to determine viable and total cell counts
- Sterile, disposable, polycarbonate snap-cap tubes
- Vortex mixer

Continued on next page

293fectin™

293fectin™ is a proprietary formulation suitable for transfecting nucleic acids into eukaryotic cells. In the FreeStyle™ 293 Expression System, use of 293fectin™ to transfect FreeStyle™ 293-F cells provides the following advantages:

- 293fectin™ demonstrates high transfection efficiency in suspension FreeStyle™ 293-F cells (cultured in FreeStyle™ 293 Expression Medium)
- DNA-293fectin™ complexes can be added directly to cells in culture medium
- It is not necessary to remove complexes or change or add medium following transfection

293fectin™ is also available separately (see page 30 for ordering information). For more information, visit **www.lifetechnologies.com** or contact Technical Support (see page 31).

Opti-MEM I® Reduced Serum Medium

Opti-MEM® I Reduced Serum Medium is included with the FreeStyle™ 293 Expression System to facilitate optimal formation of DNA-293fectin™ complexes. Opti-MEM® I is a modification of Eagle's Minimal Essential Medium, 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 29 for ordering information). For more information, visit **www.lifetechnologies.com** or contact Technical Support (see page 31).

Optimal Conditions for 30 mL Transfection

We generally perform transfection experiments in a 30 mL volume. To transfect suspension FreeStyle™ 293-F cells, we recommend using the following optimized conditions:

Final transfection volume: 30 mL

Number of cells to transfect: 3×10^7 cells (final cell density of 1×10^6 cells/mL)

Amount of plasmid DNA and MembranePro reagent: 9 µg of pEF6 expression construct and 27 µL of MembranePro Reagent

Amount of 293fectin™: 60 µL

If you are using other cells, you may want to test varying amounts of 293fectin™ (e.g. 30, 40, 50, 60, 80 µL) with 30-mL-scale transfection (9 µL plasmid DNA, 27 µL MembranePro™ Reagent) to determine the optimal transfection conditions.

Continued on next page

Transfection Procedure

Follow the procedure below to transfect suspension FreeStyle™ 293-F cells in a 30-mL volume. Remember that you may keep the cells in FreeStyle™ 293 Expression Medium during transfection. We recommend including a positive control (pCMV•SPORT-βgal, supplied with the FreeStyle™ 293 Expression System) and a negative control (no DNA, no 293fectin™) in your experiment.

1. The day before transfection (day 1), determine the number of cells you need for your experiment. For each 30 mL transfection, you need 3×10^7 viable cells in 28 mL of FreeStyle™ 293 Expression Medium.

   Tip: To transfect on day 2, seed the cells at a density of 6×10^5–7×10^5 viable cells/mL. To transfect on day 3, seed the cells at a density of 3×10^5–4×10^5 viable cells/mL.

2. On the day of transfection, transfer a small aliquot of the cell suspension to a microcentrifuge tube and determine cell viability and the amount of cell clumping. Vigorously vortex for 45 seconds to break up any cell clumps and determine total cell counts. Viability of the cells must be over 90%.

   Important: For optimal transfection results, make sure that you have a single cell suspension. It may be necessary to vortex the cells for 10 to 30 seconds.

3. Calculate the volume of cell suspension that contains the appropriate number of cells for one transfection (for each 30 mL transfection, you need 3×10^7 viable cells). Place the shaker flask containing cells in a 37°C incubator on an orbital shaker.

4. Prepare lipid-DNA complexes **for each transfection** as follows:

   a. In a sterile 15-mL tube, dilute 9 µg of purified pEF6 expression construct and 27 µg of MembranePro™ Reagent (27 µL of the supplied 1 µg/µL solution) in Opti-MEM® I Reduced Serum Medium to a total volume of 1 mL. Mix gently.

   b. In a separate sterile 15-mL tube, dilute 60 µL of 293fectin™ (mix gently before use) in Opti-MEM® I Reduced Serum Medium to a total volume of 1 mL.

   Mix the tubes gently and incubate for 5 minutes at room temperature.

   Note: Longer incubation times may result in decreased transfection efficiency.

5. After incubation, combine the diluted DNA (Step a) with the diluted 293fectin™ (Step b) to obtain a total volume of 2 mL. Mix gently.

6. Incubate the mixture for 20–30 minutes at room temperature to allow the DNA-293fectin™ complexes to form.

7. While the DNA-293fectin™ complexes are incubating, remove the cell suspension from the incubator and transfer the appropriate volume (see step 3) into each sterile, disposable 125-mL Erlenmeyer shaker flask. Add fresh, pre-warmed FreeStyle™ 293 Expression Medium up to a total volume of 28 mL for a 30 mL transfection.

Continued on next page

Scalable Production of Virus-Like Particles (VLPs) in FreeStyle™ 293-F Cells, continued

Transfection Procedure, continued

8. After the DNA-293fectin™ complex incubation is complete, add 2 mL of the complex into each shaker flask from Step 7. To the negative control flask, add 2 mL of Opti-MEM® I instead of the DNA-293fectin™ complex. Each flask should contain a total volume of 30 mL, with a final cell density of approximately 1×10^6 viable cells/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. Harvest the cells or media (if recombinant protein is secreted) at approximately 48 hours post-transfection and assay for recombinant protein expression.

Optimizing VLP Production

Expression levels may vary depending on the nature of your membrane protein; therefore, you may want to perform a time course (i.e., harvest cells or media at 24, 48, 72, and 96 hours post-transfection) to optimize expression and capture of your protein.

Scaling-Up Transfections

It is possible to perform transfections in a larger (e.g., 1 liter) volume. To transfect suspension FreeStyle™ 293-F cells in a larger volume, scale up the volume of each reagent accordingly. The table below lists suggested conditions to use when transfecting FreeStyle™ 293-F cells in a 1 liter volume. The optimized conditions to use when transfecting FreeStyle™ 293-F cells in a 30 mL volume are listed as a reference. Note that transfection conditions vary depending on the type of culture vessel used and the growth conditions of your cells; therefore, we recommend that you perform pilot studies to optimize your transfection conditions.

Transfection Volume	Total Number of Cells*	Amount of DNA	Amount of MembranePro™ Reagent	Dilution Volume†	Amount of 293fectin™	293fectin™ Dilution Volume†	Lipid/DNA Complex Volume
30 mL	3×10^7	9 µg	27 µL	to 1 mL	60 µL	to 1 mL	2 mL
1 liter	1×10^9	300 µg	900 µL	to 35 mL	2 mL	to 35 mL	70 mL

*Final cell density of 1×10^6 viable cells/mL.

†Diluted in Opti-MEM® I Reduced Serum Medium.

The efficiency of the transfection reaction may decrease as the transfection volume increases, especially if the FreeStyle™ 293-F cells are not growing as a single-cell suspension (i.e., if there is significant cell clumping).

Continued on next page

Harvesting Procedure

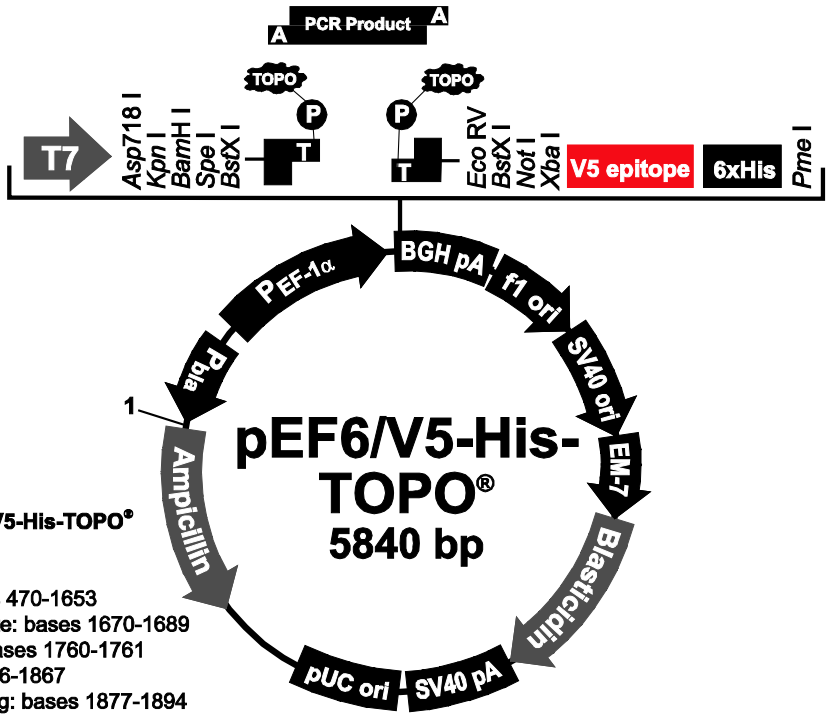
1. Transfer cultures to 50-mL conical tubes and centrifuge at 1,000 × *g* for 10 minutes in a clinical centrifuge with a swinging bucket rotor to pellet and remove the cell debris.
2. Transfer the media supernatant to fresh conical tubes and centrifuge at 2,000 × *g* for 10 minutes to ensure that all cell debris is removed.
3. Carefully recover clarified supernatant using a 10-mL pipette, leaving the last 1–2 mL of media in the tube to avoid a carry-over of cell debris.
4. Recover the VLPs using MembranePro™ Precipitation mix as described in the standard MembranePro™ protocol (see page 17).

pEF6/V5-His-TOPO® Vector

Map of pEF6/V5-His-TOPO®

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.

The pEF6/V5-His-TOPO® vector is supplied with the MembranePro™ Functional Protein Expression Kit and it is also available separately from Life Technologies (see page 29). For more information on the pEF6/V5-His-TOPO® vector, refer to the pEF6/V5-His TOPO® TA Vector Kit manual (part no. 25-0279). **The complete sequence for pEF6/V5-His-TOPO® is available for downloading at www.lifetechnologies.com or by contacting Technical Support (see page 31).**



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)

Continued on next page

pEF6/V5-His-TOPO® Vector, continued

Features of pEF6/V5-His-TOPO®

pEF6/V5-His-TOPO® (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*

Ordering Information

Additional MembranePro™ Components

Many of the components of the MembranePro™ Functional Protein Expression kits are also available separately from Life Technologies. These products are listed below. For more information, refer to **www.lifetechnologies.com** or contact Technical Support (see page 31).

Product	Amount	Cat. no.
pEF6 V5-His TOPO® TA Expression Kit	20 reactions	K9610-20
293FT Cell Line	3×10^7 cells	R700-07
Lipofectamine® 2000 Transfection Reagent	0.75 mL 1.5 mL 15 mL	11668-027 1668-019 11668-500
Opti-MEM® I Reduced Serum Medium	100 mL 500 mL	31985-062 31985-070
One Shot® TOP10 Chemically Competent *E. coli*	10 reactions 20 reactions 40 × 50 µL	C4040-10 C4040-03 C4040-06

Media, Buffers, and Reagents

We recommend the following media and buffers for culturing, passaging, maintaining, and transfecting your 293FT cell cultures. For more information on these and other cell culture products available from Life Technologies, refer to **www.lifetechnologies.com** or contact Technical Support (see page 31).

Note: Products are also available in different quantities and packaging sizes.

Product	Amount	Cat. no.
Dulbecco's Modified Eagle Medium (D-MEM)	500 mL	11965-092
Dulbecco's Modified Eagle Medium (D-MEM) high glucose with L-glutamine and sodium pyruvate	500 mL	11995-065
Opti-MEM® I Reduced Serum Medium	500 mL	31985-070
Dulbecco's Phosphate Buffered Saline (D-PBS) (1X), liquid	1,000 mL	14040-117
Phosphate Buffered Saline (PBS) pH 7.4(1X), liquid	500 mL	10010-023
MEM Non-Essential Amino Acids Solution 10 mM (100X), liquid	100 mL	11140-050
MEM Sodium Pyruvate Solution 10 mM (100X), liquid	100 mL	11360-070
L-Glutamine, 200 mM (100X), liquid	100 mL	25030-081
Fetal Bovine Serum	500 mL	16000-044
Geneticin® Selective Antibiotic, liquid	20 mL	10131-027

Continued on next page

Ordering Information, continued

Additional Products

The products listed below may be used with MembranePro™ Functional Protein Expression kits. For more information, refer to **www.lifetechnologies.com** or contact Technical Support (see page 31).

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
Countess® Automated Cell Counter (includes 50 Countess® cell counting chamber slides and 2 mL of Trypan Blue Stain)	1 unit	C10227
Trypan Blue Stain	100 mL	15250-061
β-Gal Assay Kit	80 mL	K1455-01
β-Gal Staining Kit	1 kit	K1465-01
Water, distilled	20 × 100 mL	15230-196

FreeStyle™ 293 Expression System

The products listed below may be used with MembranePro™ Functional Protein Expression kits for the scalable production of VLPs in FreeStyle™ 293-F cells. For more information, refer to **www.lifetechnologies.com** or contact Technical Support (see page 31).

Product	Amount	Cat. no.
FreeStyle™ 293 Expression System	1 kit	K9000-01
FreeStyle™ 293-F cells	1 vial (1×10^7 cells)	R790-07
FreeStyle™ 293 Expression Medium	1 liter 6 × 1 liter	12338-018 12338-026
293fectin™	1 mL	12347-019
pCMV•SPORT-βgal	25 µg	10586-014

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.

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Purchaser Notification

Information for European Customers	The 293FT Cell Line is genetically modified and carries the pUC-derived plasmid, pCMVSPORT6TAg.neo. As a condition of sale, use of this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.
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