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Growth and Maintenance of the 293FT Cell Line

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User Manual

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The 293FT Cell Line is used for the production of lentiviral stocks. The 293FT Cell Line is supplied as one vial containing 3 x 10^6 frozen cells in 1 ml of Freezing Medium. **Upon receipt, store in liquid nitrogen until use.**

Handle as potentially biohazardous material under at least Biosafety Level 2 containment. This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

Accessory Products

Accessory Products

The products listed below may be used with the 293FT Cell Line. For more information, refer to our Web site (www.invitrogen.com) or call Technical Support (see page 10).

Note: Some reagents are available in other sizes.

Item	Amount	Catalog no.
Lipofectamine™ 2000 Reagent	0.75 ml	11668-027
	1.5 ml	11668-019
Dulbecco's Modified Eagle Medium (D-MEM)	500 ml	11965-092
	1000 ml	11965-084
Fetal Bovine Serum	100 ml	16000-036
	500 ml	16000-044
10 mM MEM Non-Essential Amino Acids Solution	100 ml	11140-050
200 mM L-Glutamine	100 ml	25030-081
MEM Sodium Pyruvate Solution (100X)	100 ml	11360-070
Penicillin-Streptomycin	100 ml	15070-063
Trypsin-EDTA	100 ml	25300-054
Geneticin® Selective Antibiotic	1 g	11811-023
	5 g	11811-031
	20 ml (50 mg/ml)	10131-035
	100 ml (50 mg/ml)	10131-027
Opti-MEM® I Reduced Serum Medium	100 ml	31985-062
	500 ml	31985-070
Phosphate-Buffered Saline (PBS), pH 7.4	500 ml	10010-023
	1 L	10010-031
Trypan Blue Stain	100 ml	15250-061

Introduction

Product Information

293FT Cell Line

The 293FT Cell Line is a very suitable host for lentiviral production. The 293FT Cell Line is derived from the 293F Cell Line (see below) and stably expresses the SV40 large T antigen from the pCMVSPORT6TAg.neo plasmid. Expression of the SV40 large T antigen is controlled by the human cytomegalo-virus (CMV) promoter and is high-level and constitutive. For more information about pCMVSPORT6TAg.neo, see the **Appendix**, page 9.

Use of the Cell Line

Studies have demonstrated maximal virus production in human 293 cells expressing SV40 large T antigen (Naldini *et al.*, 1996), making the 293FT Cell Line a particularly suitable host for generating lentiviral constructs using the ViraPower™ Lentiviral Expression System available from Invitrogen (Catalog nos. K4950-00 and K4960-00).

Parental Cell Lines

The 293 Cell Line is a permanent line established from primary embryonal human kidney transformed with sheared human adenovirus type 5 DNA (Graham *et al.*, 1977; Harrison *et al.*, 1977). The E1A adenovirus gene is expressed in these cells and participates in transactivation of some viral promoters, allowing these cells to produce very high levels of protein.

The 293-F Cell Line available from Invitrogen (Catalog no. 11625) is a fast-growing variant of the 293 cell line, and was originally obtained from Robert Horlick at Pharmacopeia.

Antibiotic Resistance

293FT cells stably express the neomycin resistance gene from pCMVSPORT6TAg.neo and should be maintained in medium containing Geneticin® at the concentration listed below. Expression of the neomycin resistance gene in 293FT cells is controlled by the SV40 enhancer/promoter.

Methods

General Information

General Cell Handling

Follow the general guidelines below to grow and maintain 293FT cells.

- **Make sure that all solutions and equipment that come in contact with the cells are 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.
- For general maintenance of cells, pass 293FT cells when they are > 80% confluent. Avoid overgrowing cells before passaging.
- Maintain 293FT cells in complete medium containing 500 µg/ml Geneticin®.
- Use trypan blue exclusion to determine cell viability. Log phase cultures should be >90% viable.
- When thawing or subculturing cells, transfer cells into medium warmed to room temperature.
- Cells should be at the appropriate confluence and at greater than 90% viability prior to transfection (see page 8).

Before Starting

Be sure to have the following solutions and supplies available:

- 15 ml sterile, conical tubes
- Appropriate sized tissue culture flasks and pipettes
- Complete medium (see page 3)
- 50 mg/ml Geneticin®
- Phosphate-Buffered Saline (PBS; Invitrogen, Catalog no. 10010-023)
- Reagents for counting cells
- Trypsin/versene (EDTA) solution or other trypsin solution
- Freezing Medium (see pages 3 and 7)
- Table-top centrifuge
- Cryovials (if needed)

Continued on next page

General Information, continued

Media for 293FT Cells

The table below lists the recommended complete medium, freezing medium, and antibiotic concentration required to maintain and culture the 293FT Cell Line.

Note: Fetal bovine serum should not be heat-inactivated for use with the 293FT Cell Line.

Complete Medium	[Antibiotic]	Freezing Medium
D-MEM (high glucose) 10% fetal bovine serum (FBS) 0.1 mM MEM Non-Essential Amino Acids (NEAA) 6 mM L-glutamine 1 mM MEM Sodium Pyruvate 1% Pen-Strep (optional)	500 µg/ml Geneticin®	90% complete medium 10% DMSO

Note

D-MEM already contains 4 mM L-glutamine, which is enough to support cell growth of the 293FT Cell Line. However, since L-glutamine slowly decays over time, the complete medium needs to be supplemented with 2 mM L-glutamine. This will ensure that the concentration of L-glutamine in complete medium will not get too low over time due to its slow degradation.

Note: 293FT cells grow well in 6 mM L-glutamine, but higher concentrations of L-glutamine may reduce growth.

Preparing Medium

Prepare the complete D-MEM medium containing 10% FBS supplemented with 0.1 mM MEM Non-Essential Amino Acids, 1 mM sodium pyruvate and 2 mM L-glutamine as described below using reagents

Perform all steps in a tissue culture hood under sterile conditions.

1. Remove 100 ml D-MEM from 1 L D-MEM bottle and replace with 100 ml FBS.
2. To the bottle of medium, add the following:

200 mM L-Glutamine (100X)	10 ml
10 mM MEM Non-Essential Amino Acids (100X)	10 ml
100 mM MEM Sodium Pyruvate (100X)	10 ml
Optional: Penicillin-Streptomycin (100X)	10 ml

3. Filter sterilize the medium using 0.45 µm filtration device.
4. Store the complete medium at 4°C until use. The medium is stable for 6 months at 4°C (avoid introducing any contamination into the medium).
5. To an aliquot of the complete medium, add Geneticin® to prepare complete medium with 500 µg/ml Geneticin®.

Thawing Cells

Introduction

The 293FT Cell Line is supplied in a vial containing 3 x 10^6 cells in 1 ml of Freezing Medium. Store frozen 293FT cells in liquid nitrogen until ready to use.

Handle as potentially biohazardous material under at least Biosafety Level 2 containment. This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

Thawing Cells

Use the following procedure to thaw 293FT cells to initiate cell culture. Thaw cells in prewarmed, complete medium **without** Geneticin®.

1. Remove the vial of frozen cells from liquid nitrogen and thaw quickly in a 37°C water bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol, and transfer the cells to a sterile 15 ml tube containing PBS. Briefly centrifuge the cells at 150-200 x g and resuspend the cells in 2 ml complete medium without Geneticin®.
3. Transfer the cells to T-75 cm^2 flask containing 10 ml of complete medium without Geneticin®.
4. Incubate the flask overnight at 37°C for allowing the cells to attach to the bottom of the flask.
5. The next day, aspirate off the medium and replace with fresh, complete medium containing 500 µg/ml Geneticin®.
6. Incubate the cells and check them daily until the cells are 80-90% confluent.
7. Proceed to **Subculturing Cells**, next page.

We recommend subculturing cells for a minimum of 3 passages after thawing before use in other applications.

Don't add Geneticin to cells that are coming from Cryogenic Storage

Subculturing Cells

Introduction

Follow the recommendations and procedures in this section to subculture 293FT cells. Maintain cells as adherent monolayer cultures in complete medium containing 500 µg/ml Geneticin®.

Subculturing Conditions

Use the following recommended conditions to subculture 293FT cells. For a procedure to subculture cells, see below.

Parameter	Recommended Condition
Cell density	> 5 x 10^5 viable cells/ml (> 80% confluent)
Culture vessel	T-75 cm^2 to T-162 cm^2 disposable sterile T-flasks. Dilute cells in a total working volume of 15-20 ml for T-75 cm^2 flasks and 40-50 ml for T-162 cm^2 flasks
Seeding density	2 to 5 x 10^4 viable cells/cm^2
Incubation conditions	37°C incubator with a humidified atmosphere of 5-10% CO_2 in air; loosen caps to allow for oxygenation/aeration

Determining Viability and Cell Density

Follow the procedure below to determine viable and total cell counts using the trypan blue exclusion method.

1. Transfer a small aliquot of the cell suspension to a microcentrifuge tube and dilute the cells such that the total number of cells counted will not be less than 100 or more than 1,000.
2. To 1 ml of the diluted cell suspension, add 100 µl Trypan Blue Stain (0.4%) solution. Gently aspirate to mix.
3. Record the dilution factor. The dilution factor equals the total volume (amount of cell suspension and amount of trypan blue) divided by the amount of cell suspension.
4. Incubate the cells with the trypan blue solution for 1-2 minutes.
5. Count all cells (including the blue cells) using a Coulter Counter or manually using a hemocytometer chamber.
6. To calculate the total cells per ml in suspension, multiply the total count by the dilution factor.
7. To determine the viability, count only the blue cells. Calculate the % viability:

[1.00 - (Number of blue cells ÷ Number of total cells)] x 100

8. Cell viability should be at least 95% for healthy log-phase cultures.

Continued on next page

Subculturing Cells, continued

Subculturing Cells

Use this procedure to subculture 293FT cells grown in a T-75 cm^2 flask. If you are using other-sized flasks, scale the reagent volumes accordingly.

1. Remove all medium from the flask and wash the cells once with 10 ml PBS to remove excess medium and serum. Serum contains inhibitors of trypsin.
2. Add 2 ml of trypsin/versene (EDTA) solution to the monolayer and incubate 1-5 minutes at room temperature until cells detach. Check the cells under a microscope and confirm that most of the cells have detached. If cells are still attached, incubate a little longer until most of the cells have detached.
3. Add 8 ml complete medium containing Geneticin® and transfer the cell suspension to a 15 ml sterile, conical tube.
4. Determine viable and total cell counts (see procedure above).
5. Seed cells at the recommended density (see table on previous page), diluting in pre-warmed complete medium containing 500 µg/ml Geneticin®. Incubate flasks as recommended (see table on previous page).
6. Maintain cells as adherent monolayer cultures in complete medium containing 500 µg/ml Geneticin®.
7. For the transfection protocol, you will need 6 x 10^6 293FT cells for each sample (page 8).

Freezing Cells

Introduction

Once you have established the cells, we recommend freezing some cells for future use as described below.

- Freeze cells at a density of **at least** 3 x 10^6 viable cells/ml.
- Use a freezing medium composed of 90% complete medium and 10% DMSO. Prepare freezing medium immediately before use. Filter-sterilize the freezing medium and store at +4°C until use. Discard any remaining freezing medium after use.

Freezing Cells

Before starting, label cryovials and prepare freezing medium (see above). Keep the freezing medium on ice.

1. Culture the desired quantity of 293FT cells to 70-90% confluency.
2. Remove the cells from the tissue culture flask(s) following Steps 1-3, **Subculturing Cells**, page 6.
3. Determine viable and total cell counts (see procedure on page 5) and calculate the volume of freezing medium required to yield a final cell density of ≥ 3 x 10^6 cells/ml.
4. Prepare the required volume of freezing medium (see above).
5. Centrifuge the cells suspension (from Step 2) at 250 x g for 5 minutes in a table top centrifuge at room temperature. Carefully aspirate off the medium and resuspend the cell pellet in the pre-determined volume of chilled freezing medium.
6. Dispense aliquots of this suspension (frequently mixing to maintain a homogeneous cell suspension) into cryovials according to manufacturer's specifications.
7. Freeze cells in an automated, controlled-rate freezing apparatus or using a manual method following standard procedures. For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.
8. Transfer vials to liquid nitrogen storage.

Note: You may check the viability and recovery of frozen cells 24 hours after storing cryovials in liquid nitrogen by following the procedure outlined in **Thawing Cells**, page 4.

Don't add Geneticin to cells that are for Cryogenic Storage

Transfecting Cells

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. Lipofectamine™ 2000 is recommended, but other transfection reagents are suitable. Lipofectamine™ 2000 is available from Invitrogen (see page vi for ordering information).

Transient Transfection

The 293FT Cell Line may be transiently transfected with any plasmid. General guidelines are provided below.

- Make sure that cells are healthy at the time of plating. Overgrowth of cells prior to passaging can compromise their transfection efficiency.
- On the day before transfection, plate cells such that they will be at the appropriate confluence at the time of transfection (see manufacturer's recommendations for the transfection reagent you are using). **Example:** If you are using Lipofectamine™ 2000 as a transfection reagent, plate cells such that they will be 90-95% confluent at the time of transfection.
- Transfect your plasmid construct into the 293FT Cell Line using the method of choice (see above).
- After transfection, add fresh growth medium containing 500 µg/ml Geneticin® and allow the cells to recover for 24-48 hours before proceeding to assay for expression of your gene of interest.

Generating Stable Cell Lines

293FT cells can be used as hosts to generate a stable cell line expressing your gene of interest from most plasmids (see **Note** below). Remember that the introduced plasmid must contain a selection marker other than neomycin resistance. Stable cell lines can then be generated by transfection and dual selection with Geneticin® and the appropriate selection agent.

Note

Since 293FT cells stably express the SV40 large T antigen, we **do not** recommend generating stable cell lines with plasmids that contain the SV40 origin of replication.

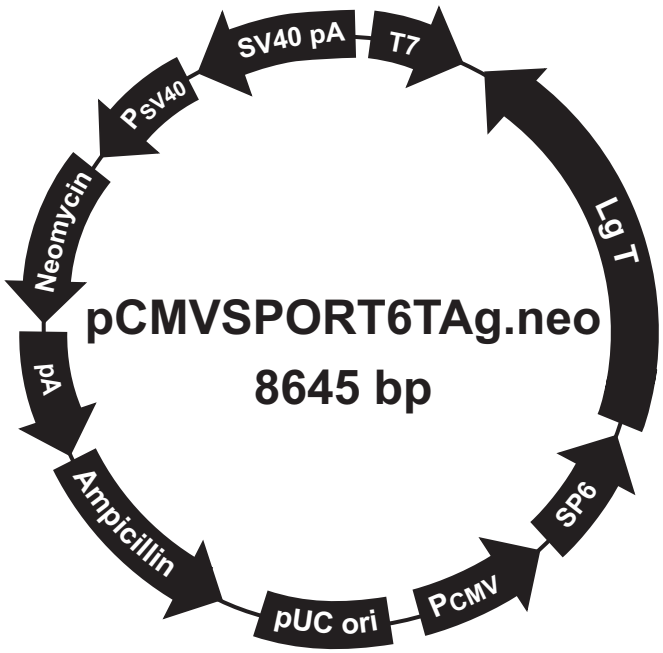
Appendix

Map of pCMVSPORT6TAg.neo

Description

The pCMVSPORT6TAg.neo plasmid is derived from pCMVSPORT6, which has been modified to include the following features:

- The neomycin resistance gene for stable selection in mammalian cells (Southern & Berg, 1982). Expression of the neomycin resistance gene is controlled by the SV40 early enhancer/promoter from which the SV40 origin of replication has been removed.
- The gene encoding the SV40 large T antigen to facilitate optimal virus production (*e.g.* Invitrogen's ViraPower™ Lentiviral Expression System) and to permit episomal replication of plasmids containing the SV40 early promoter and origin. Expression of the SV40 large T antigen is controlled by the human cytomegalovirus (CMV) promoter.



Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
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For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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Purchaser Notification

Introduction

Use of the 293FT Cell Line is covered under the licenses detailed below.

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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