Invitrogen™
life technologies

Instruction Manual

Growth and Maintenance of the 293A Cell Line

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Important Information

Shipping/ Storage

The 293A Cell Line is shipped on dry ice. Upon receipt, store in liquid nitrogen.

Contents

The 293A Cell Line is supplied as one vial containing 3×10^6 frozen cells in 1 ml of Freezing Medium.

Product Qualification

Each lot of cells is tested for cell growth and viability post-recovery from cryopreservation. Master Cell Banks are screened for viruses, mycoplasma, and sterility. Flat morphology is confirmed by visual inspection.

Information for European Customers

The 293A cell line is genetically modified and includes human adenovirus type 5 DNA. As a condition of sale, 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.

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 293A Cell Line. For more information, refer to our Web site (www.invitrogen.com) or call Technical Service (see page 9).
Note: Some reagents are available in other sizes.

Item	Amount	Catalog no.
Dulbecco's Modified Eagle Medium (D-MEM)	500 ml	11965-092
Fetal Bovine Serum	500 ml	16000-044
10 mM MEM Non-Essential Amino Acids Solution	100 ml	11140-050
200 mM L-Glutamine	100 ml	25030-081
Penicillin-Streptomycin	100 ml	15070-063
Trypsin-EDTA	100 ml	25300-054
Versene-EDTA	100 ml	15040-066
Lipofectamine™ 2000 Reagent	0.75 ml	11668-027
	1.5 ml	11668-019
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

Overview

Introduction

The 293A Cell Line is a subclone of the 293 Cell Line (see below) and has a relatively flat morphology.

Use of the Cell Line

The flat morphology of 293A cells make them particularly suitable for screening adenoviral constructs using the ViraPower™ Adenoviral Gateway™ Expression Kit (Catalog no. K4930-00) and the ViraPower™ Adenoviral Promoterless Gateway™ Expression Kit (Catalog no. K4940-00), available from Invitrogen.

Parental Cell Line

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 genes encoded by the E1 region of adenovirus (E1a and E1b) are expressed in these cells and participate in transactivation of some viral promoters, allowing these cells to produce very high levels of protein. E1 also complements the E1-deletion in recombinant adenoviral vectors, allowing viral replication.

Media for 293A Cells

The table below lists the recommended complete medium and freezing medium required to maintain and culture the 293A Cell Line. **Note:** FBS does not need to be heat-inactivated for use with the 293A Cell Line.

Complete Medium	Freezing Medium
D-MEM (high glucose) 10% fetal bovine serum (FBS) 0.1 mM MEM Non-Essential Amino Acids (NEAA) 2 mM L-glutamine 1% Pen-Strep (optional)	90% complete medium 10% DMSO

Methods

General Information

General Cell Handling

Follow the general guidelines below to grow and maintain 293A 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 293A cells when they are > 80-90% confluent. Avoid overgrowing cells before passaging.
- 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).

As with other human cell lines, when working with 293A cells, handle as potentially biohazardous material under Biosafety Level 2 (BL-2) containment.

For more information on BL-2 guidelines and adenovirus handling, please refer to *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed., published by the Centers for Disease Control, or see the following Web site: www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

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General Information, continued

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
- Cryovials (if needed)
- Phosphate-Buffered Saline (PBS; Invitrogen, Catalog no. 10010-023)
- Trypsin/versene (EDTA) solution or other trypsin solution
- Reagents for counting cells
- Freezing Medium (see pages 1 and 7)
- Table-top centrifuge

Thawing Cells

Introduction

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

Thawing Cells

Use the following procedure to thaw 293A cells to initiate cell culture.

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 T-75 flask containing 12 ml of complete medium.
3. Incubate the flask at 37°C for 2-4 hours to allow the cells to attach to the bottom of the flask.
4. Aspirate off the medium and replace with 12 ml of fresh, complete medium.
5. Incubate the cells in a 37°C incubator containing a humidified atmosphere of 5-10% CO_2 in air. Loosen caps of flasks to allow oxygenation/aeration. Check the cells daily until they are 80-90% confluent.
6. Proceed to **Subculturing Cells**, next page.

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

Subculturing Cells

Introduction

Follow the recommendations and procedures in this section to subculture 293A cells.

Subculturing Conditions

Use the following recommended conditions to subculture 293A cells. For a procedure to subculture cells, see the next page.

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.

1. Transfer a small aliquot of the cell suspension to a microcentrifuge tube.
2. Determine viability and the amount of cell clumping using the trypan blue exclusion method.
3. Vigorously vortex cells for up to 40 seconds to break up cell clumps.
4. Determine cell density electronically using a Coulter Counter or manually using a hemocytometer chamber.

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Subculturing Cells, continued

Passaging Cells

Use this procedure to subculture 293A cells grown in a T-75 cm^2 flask. If you are using other-sized flasks, scale the reagent volumes up or down 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 5 ml of trypsin/versene (EDTA) solution to the monolayer and incubate 1 to 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 5 ml of complete medium and transfer the cell suspension to a 15 ml sterile, conical tube.
4. Determine viable and total cell counts (see procedure on the previous page).
5. Seed cells at the recommended density (see table on the previous page), diluting in pre-warmed complete medium. Incubate flasks as recommended (see table on the previous page).

Freezing Cells

Introduction

When freezing the 293A Cell Line, we recommend the following:

- 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 293A 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 cell 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.

Transfecting Cells

Transfection Methods

The 293A Cell Line is generally amenable to transfection using standard methods including lipid-mediated transfection (Felgner *et al.*, 1989; Felgner and Ringold, 1989), calcium phosphate precipitation (Chen and Okayama, 1987; Wigler *et al.*, 1977), and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). We typically use cationic lipid-based transfection reagents to transfect 293A 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 293A 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 293A Cell Line using the method of choice (see above).
- After transfection, add fresh medium 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

293A cells can be used as hosts to generate a stable cell line expressing your gene of interest from most plasmids. Stable cell lines can then be generated by transfection and selection with the appropriate selection agent.

Appendix

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).

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Technical Service, continued

MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

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