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Growth and Maintenance of the 293A Cell Line

USER GUIDE

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Revision	Date	Description
A.0	06 July 2016	Rebrand, update cell density
B	31 March 2003	Baseline for this revision

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Product information

Product description

The 293A Cell Line is a subclone of the 293 Cell Line and has a relatively flat morphology. The 293 Cell Line is a permanent line established from primary embryonal human kidney transformed with sheared human adenovirus type 5 DNA. 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.

Contents and storage

Contents	Amount	Storage
293A Cell Line	1.1 x 10^7 cells/mL	Shipped at on dry ice. Store in liquid nitrogen.

Required materials not supplied

- 15 mL sterile, conical tubes
- Appropriate sized tissue culture flasks and pipettes
- Complete medium
- Cryovials (if needed)
- Phosphate-Buffered Saline
- Trypsin/versene (EDTA) solution or other trypsin solution
- Cell counting reagents
- Freezing medium
- Table-top centrifuge

Methods

Procedural guidelines

- 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.
- Transfer cells into medium warmed to room temperature when thawing or subculturing cells.
- Cells should be at the appropriate confluence and at greater than 90% viability prior to transfection.
- **IMPORTANT!** As with other human cell lines, when working with 293A cells, handle as potentially biohazardous material under Biosafety Level 2 (BL-2) containment.

Recommended complete medium and freezing medium

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

Thaw cells

The 293A Cell Line is supplied in a vial containing 1.1×10^7 cells in 1 mL of Freezing Medium. Store frozen 293A cells in liquid nitrogen until ready to use.

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.
   a. Loosen caps of flasks to allow oxygenation/aeration.
6. Check the cells daily until they are 80–90% confluent.
   We recommend subculturing cells for a minimum of 3 passages after thawing before use in other applications.

Subculture cells

Recommended subculture conditions

Parameter	Recommended condition
Cell density	> 5×10^5 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	$2–5 \times 10^4$ 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

Determine viability and cell density

1. Transfer a small aliquot of the cell suspension to a microcentrifuge tube.
2. Use trypan blue exclusion method to determine viability and the amount of cell clumping.
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.

Passage 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.
   a. Check the cells under a microscope and confirm that most of the cells have detached.
   b. 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 “Recommended subculture conditions“ on page 6.
5. Seed cells at the recommended density, diluting in pre-warmed complete medium, then incubate flasks as recommended . “Recommended subculture conditions“ on page 6

Freeze cells

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

- – Freeze cells at a density of at least 3 × 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.

Label cryovials and prepare freezing medium. 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, “Passage cells“ on page 7.
3. Determine viable and total cell counts “Recommended subculture conditions“ on page 6 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.
6. Carefully aspirate off the medium and resuspend the cell pellet in the pre-determined volume of chilled freezing medium.

7. Dispense aliquots of this suspension (frequently mixing to maintain a homogeneous cell suspension) into cryovials according to manufacturer's specifications.

8. 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.

9. 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 "Thaw cells" on page 6.

Transfect cells

Transfection methods

The 293A Cell Line is generally amenable to transfection using standard methods including lipid-mediated transfection, calcium phosphate precipitation, and electroporation.

Transient transfection guidelines

The 293A Cell Line can be transiently transfected with any plasmid.

- Make sure that cells are healthy at the time of plating. Overgrowth of cells prior to passaging can compromise their transfection efficiency.
- Make sure that cells are healthy at the time of plating. Overgrowth of cells prior to passaging can compromise their transfection efficiency.
- Transfect your plasmid construct into the 293A Cell Line using the method of choice.
- 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.

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.

Accessory products

Accessory products

All materials are available through **thermofisher.com**

Item	Cat. No.
Dulbecco's Modified Eagle Medium (DMEM)	11965-092
Fetal Bovine Serum	16000-044
10 mM MEM Non-Essential Amino Acids Solution	11140-050
200 mM L-Glutamine	25030-081
Penicillin-Streptomycin	15070-063
Trypsin-EDTA	25300-054
Versene-EDTA	15040-066
Lipofectamine™ 2000 Reagent	11668-027
Opti-MEM™ I Reduced Serum Medium	31985-062
Phosphate-Buffered Saline	10010-031

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Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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