AAV-293 Cells

Catalog #240073

Shipping: The AAV-293 cells are shipped on dry ice.

Storage: Place the cells in liquid nitrogen immediately upon arrival.

Introduction

Stratagene recommends preparing adeno-associated recombinant virus stocks using the AAV-293 cell line. AAV-293 cells are derived from the commonly used HEK293 cell line, but produce higher viral titers. HEK293 cells are human embryonic kidney cells that have been transformed by sheared adenovirus type 5 DNA.[1] AAV-293 cells, like HEK293 cells, produce the adenovirus E1 gene in *trans,* allowing the production of infectious adeno-associated virus particles when cells are co-transfected with the three AAV Helper-Free System plasmids (an ITR-containing plasmid, pAAV-RC, and the E1-deleted pHelper plasmid).

Materials Provided

Materials Provided	Quantity
AAV-293 cells (provided in 1-ml DMEM + 40% FBS + 10% DMSO)	1×10^6 cells

Additional Materials Required

Complete DMEM Growth Medium [Invitrogen Life Technologies (Gibco) DMEM Catalog #11995]
(See *Preparation of Media and Reagents*)
Tissue culture plasticware and reagents

AAV-293 Cell Culture Guidelines

Notes *All procedures must be performed using sterile technique in a laminar flow hood. For general information on mammalian cell culture and sterile technique, see reference 2.*

AAV-293 cells do not adhere well to tissue culture dishes and have a tendency to clump. When exchanging solutions, gently pipette down the side of the dish and not directly onto the cells to prevent disruption of the cell monolayer.

It is important to prepare a liquid nitrogen stock of early passage cell aliquots for long-range experiments.

Establishing AAV-293 Cultures from Frozen Cells

1. Place 10 ml of complete DMEM growth medium (see *Preparation of Media and Reagents*) in a 15-ml conical tube.
2. Thaw the frozen cryovial of cells within 1–2 minutes by gentle agitation in a 37°C water bath. Remove the cryovial from the water bath and decontaminate the cryovial by wiping the surface of the vial with 70% (v/v) ethanol (at room temperature).
3. Transfer the thawed cell suspension to the conical tube containing 10 ml of growth medium.
4. Collect the cells by centrifugation at 200 × *g* for 3 minutes at room temperature. Remove the growth medium by aspiration.
5. Resuspend the cells in the conical tube in 5 ml of fresh growth medium by gently pipetting up and down.
6. Transfer the 5 ml of cell suspension to a 75-cm^2 tissue culture flask containing 10 ml of fresh growth medium. Place the cells in a 37°C incubator at 5% CO_2.
7. Monitor cell density daily. Cells should be passaged when the culture reaches 50% confluence. Proceed to either *Passaging of AAV-293 Cells* or *Preparation of an AAV-293 Cell Liquid Nitrogen Stock*.

Passaging of AAV-293 Cells

Cells should be passaged when the cell monolayer reaches 50% confluence. If cell confluence exceeds 70%, AAV-293 cells may lose the increased virus production feature. The following protocol passages cells from 75-cm^2 tissue culture flasks.

1. Prewarm the complete DMEM growth medium and trypsin-EDTA solution to 37°C in a water bath.
2. Remove the growth medium by aspiration. Wash cells once with 10 ml of phosphate-buffered saline (PBS; see *Preparation of Media and Reagents*).
3. Trypsinize cells for 1–3 minutes in 5 ml of trypsin-EDTA solution (see *Preparation of Media and Reagents*).

   Note *Incubate the cells in the trypsin-EDTA solution for the minimum time required to release adherent cells from the flask. This process may be monitored using an inverted microscope. Excess trypsinization may damage or kill the cells.*

4. Dilute the cells with 5 ml of growth medium to inactivate the trypsin.
5. Transfer 2 ml of the cell suspension and add 28 ml of fresh growth medium to each of 5 fresh 175-cm^2 tissue culture flasks. Place the cells in a 37°C incubator at 5% CO_2. Monitor cell density daily. Cell confluence should be maintained at 50%.

Preparation of an AAV-293 Cell Liquid Nitrogen Stock

When growing cells for the production of an AAV-293 liquid nitrogen stock, cultures should be maintained at ≤50% confluence. The following protocol prepares frozen stocks from 175-cm^2 tissue culture flasks.

Notes *AAV-293 cells grown at high confluence may lose the increased virus production feature. It is especially important to maintain cells propagated to establish a liquid nitrogen stock at ≤50% confluence to ensure the integrity of the stock.*

Feed the cells one day prior to preparing liquid nitrogen stocks to improve viability.

1. Prewarm the complete DMEM growth medium, trypsin-EDTA solution, and freezing medium to 37°C in a water bath.
2. Collect cells from a healthy, log-phase culture. Remove the culture medium by aspiration. Wash cells with 10 ml of PBS. Trypsinize cells for 1–3 minutes in 10 ml of trypsin-EDTA solution.

   Note *Incubate the cells in the trypsin-EDTA solution for the minimum time required to release adherent cells from the flask. This process may be monitored using an inverted microscope. Excess trypsinization may damage or kill the cells.*
3. Dilute the cells with 10 ml of growth medium to inactivate the trypsin. Transfer the cell suspension into a sterile 50-ml conical tube. Count the cells present in an aliquot of the resuspension using a hemocytometer.
4. Collect the cells by centrifugation at 200 × *g* for 10 minutes at room temperature. Remove the growth medium by aspiration.
5. Resuspend the cell suspension to 1×10^6 cells/ml in freezing medium (see *Preparation of Media and Reagents*; do not include antibiotics), then dispense 1-ml aliquots of the suspension into 2-ml cryovials.
6. Freeze the cell aliquots gradually by placing the vials in a Styrofoam® container and then placing the container in a –80°C freezer overnight.
7. Transfer the vials of frozen cells to liquid nitrogen for long-term storage.

Preparation of Media and Reagents

Phosphate-Buffered Saline (PBS) 137 mM NaCl 2.6 mM KCl 10 mM Na_2HPO_4 1.8 mM KH_2PO_4 Adjust the pH to 7.4 with HCl	**Freezing Medium (100 ml)** 50 ml DMEM (containing 4.5 g/L glucose, 110 mg/L sodium pyruvate and 2 mM L-glutamine) 40 ml heat-inactivated fetal bovine serum 10 ml dimethylsulfoxide (DMSO) Filter sterilize
Complete DMEM Growth Medium DMEM (containing 4.5 g/L glucose, 110 mg/L sodium pyruvate, and 2 mM L-glutamine) supplemented with 10% (v/v) heat-inactivated fetal bovine serum and 2 mM of L-glutamine	**Trypsin-EDTA Solution** 0.53 mM tetrasodium ethylenediamine-tetraacetic acid (EDTA) 0.05% trypsin

References

1. Graham, F. L., Smiley, J., Russell, W. C. and Nairn, R. (1977) *J Gen Virol* 36(1):59–74.
2. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G. *et al.* (1987). *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.

Endnotes

Styrofoam® is a registered trademark of Dow Chemical.

Quality Control Testing

This cryovial contains at least 1.0×10^6 AAV-293 cells as determined by morphology, trypan-blue dye exclusion, and viable cell count. The AAV-293 cells are free of microbial contamination as determined by sterility culture testing in mTGE and YM broth, and by PCR for detection for mycoplasma.

Limited Product Warranty