AD-293 Cells

Catalog #240085

Storage: Place in liquid nitrogen immediately upon arrival.

INTRODUCTION

Stratagene's AD-293 cell line is a derivative of the commonly used HEK293 cell line, with improved cell adherence and plaque formation properties. HEK293 cells are human embryonic kidney cells transformed by sheared adenovirus type 5 DNA.[1] AD-293 cells, like HEK293 cells, produce the adenovirus E1 gene in *trans,* allowing the production of infectious virus particles when cells are transfected with E1-deleted adenovirus vectors such as the pAdEasy™-1 vector. Standard HEK293 cells do not adhere well to tissue culture dishes, hindering adherent cell culture and plaque assay procedures. AD-293 cells demonstrate improved adherence to tissue culture dishes, making AD-293 cell monolayers less susceptible to disruption during cell passaging and plaque assays.

MATERIALS PROVIDED

Material Provided	Quantity
AD-293 cells (Catalog #240085; provided in 1-ml DMEM + 40% FBS + 10% DMSO)	1×10^6 cells

ADDITIONAL MATERIALS REQUIRED

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

AD-293 CELL CULTURE GUIDELINES

Notes *Despite the improved adherence of AD-293 cells, it is important to minimize monolayer disruption during passaging and plaque assays by gently pipetting liquids down the side of the culture dish instead of pipetting directly onto the cells.*

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.

AD-293 cells may be passaged up to 30 times (with the supplied cells defined as passage number one). It is important to prepare a liquid nitrogen stock of early passage cell aliquots for long-range experiments.

Establishing AD-293 Cultures from Frozen Cells

1. Place 10 ml of growth medium (see *Preparation of Media and Reagents*) in a 15-ml conical tube.
2. Thaw the frozen cryovial of cells within 40–60 seconds by gentle agitation in a 37°C water bath. Remove the cryovial from the water bath and decontaminate the cryovial by immersing it in 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 5 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.
6. Add 10 ml of growth medium to a 75-cm^2 tissue culture flask. Transfer the 5 ml of cell suspension to the same tissue culture flask. Place the cells in a 37°C incubator at 5% CO_2.
7. Monitor cell density daily. Cells should be passaged when the culture is at ≤50% confluency. Proceed to either *Preparation of an AD-293 Cell Liquid Nitrogen Stock* or *Passaging of AD-293 Cells*.

Preparation of an AD-293 Cell Liquid Nitrogen Stock

1. When growing cells for the production of an AD-293 liquid nitrogen stock, cultures should be maintained at ≤50% confluence.

   Note *AD-293 cells grown at high confluence may lose the increased adherence phenotype. It is especially important to maintain cells propagated to establish a liquid nitrogen stock at ≤50% confluence to ensure the integrity of the stock.*

2. Collect cells from a healthy, log-phase culture. Remove the culture medium by aspiration. Trypsinize cells for 1–3 minutes in 1.5-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.*

3. Dilute the cells with 8.5 ml of growth medium. The serum in the medium inactivates the trypsin. Transfer the suspension to a 15-ml conical tube, then collect the cells by centrifugation at 600 × *g* for 5 minutes at room temperature.
4. Remove the medium by aspiration. Resuspend the cell pellet in a minimal volume of growth medium (containing 10% fetal bovine serum). Count the cells present in an aliquot of the resuspension using a hemocytometer.
5. Dilute the cell suspension to 1×10^6 cells/ml in freezing medium (see *Preparation of Media and Reagents*), 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.

Passaging of AD-293 Cells

When the cell monolayer is at ≤50% confluency, AD-293 cells should be split at a 1:10 ratio.

Note *If cell confluence exceeds 50% when passaged, AD-293 cells may lose the increased adherence phenotype.*

1. Remove the growth medium by aspiration. Wash cells once with 10 ml of phosphate-buffered saline (see *Preparation of Media and Reagents*).
2. Trypsinize cells for 1–3 minutes in 1.5-ml of Trypsin-EDTA solution.
3. Dilute the cells with 8.5 ml of growth medium to inactivate the trypsin.
4. Transfer 1 ml of the cell suspension to a fresh 75-cm^2 tissue culture flask and add 9 ml fresh growth medium. Place the cells in a 37°C incubator at 5% CO_2. Monitor cell density daily.

PREPARATION OF MEDIA AND REAGENTS

Growth Medium DMEM (containing 4.5 g/L glucose and 110 mg/L sodium pyruvate and 2 mM L-glutamine), supplemented with 10% (v/v) heat-inactivated fetal bovine serum	**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
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	**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.
AdEasy is a trademark of Johns Hopkins University.

QUALITY CONTROL TESTING

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

LIMITED PRODUCT WARRANTY