AAV-HT1080 Cells

Catalog #240109

Shipping: The AAV-HT1080 cells are shipped on dry ice.

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

Introduction

Stratagene recommends titering adeno-associated recombinant virus stocks using the AAV-HT1080 cells, a human fibrosarcoma cell line. Stratagene has found the AAV-HT1080 cells to be more permissible to AAV infection than other cells lines, such as 293 cells, and thus able to give more accurate viral titers. In addition, AAV titering protocols using AAV-HT1080 cells have been optimized and are provided in Stratagene's *AAV-Helper Free System Instruction Manual* (Catalog #240071).

Materials Provided

Materials Provided	Quantity
AAV-HT1080 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-HT1080 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 1.*

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

Establishing AAV-HT1080 Cultures from Frozen Cells

1. Place 10 ml of complete DMEM growth medium[§] 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 70–80% confluence. Proceed to either *Passaging of AAV-HT1080 Cells* or *Preparation of an AAV-HT1080 Cell Liquid Nitrogen Stock*.

Passaging of AAV-HT1080 Cells

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).[§]
3. Trypsinize cells for 1–3 minutes in 5 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.*

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 70–80%.

[§] See *Preparation of Media and Reagents.*

Preparation of an AAV-HT1080 Cell Liquid Nitrogen Stock

The following protocol prepares frozen stocks from 175-cm^2 tissue culture flasks.

Note *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. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G. *et al.* (1987). *Current Protocols in Molecular Biology*. John Wiley and 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-HT1080 cells as determined by morphology, trypan-blue dye exclusion, and viable cell count. The AAV-HT1080 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