293AAV Cell Line

CATALOG NUMBER: AAV-100

STORAGE: Liquid nitrogen
Note: For best results begin culture of cells immediately upon receipt. If this is not possible, store at -80ºC until first culture. Store subsequent cultured cells long term in liquid nitrogen.

QUANTITY & CONCENTRATION: 1 mL, 1 x 10^6 cells/mL in 90% complete medium, 10% DMSO

Background

Adeno-associated virus (AAV) belongs to the family of Parvoviridae, a group of viruses among the smallest of single-stranded and non-enveloped DNA viruses. There are nine different AAV serotypes reported to date. AAV can infect both dividing and non-dividing cells and can be maintained in the human host cell, creating the potential for long-term gene transfer. Recombinant AAV-2 is the most common serotype used in gene delivery, and can be produced at high titers with a helper virus or Cell Biolabs' AAV Helper-Free System in 293 cells.

The 293AAV Cell Line is a permanent line established from primary embryonic human kidney transformed with 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 viral promoters, allowing these cells to produce high levels of protein.

293AAV is derived from the parental 293 cell line, through cloning and multiple rounds of testing, 293AAV is specifically selected for a high level of AAV production in a helper-free system. It offers several advantages over the regular 293 cells:

- Larger cell surface area resulting higher transfection and better yield of AAV.
- Flattened morphology.
- Firm attachment to culture plate, ideal for large scale culture and AAV production.

Quality Control

This cryovial contains at least 1.0×10^6 293AAV cells as determined by morphology, trypan-blue dye exclusion, and viable cell count. The 293AAV cells are tested free of microbial contamination.

Medium

1. Culture 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)
2. Freeze Medium: 90% complete medium, 10% DMSO

Methods

Establishing 293AAV Cultures from Frozen Cells

1. Place 10 mL of complete DMEM growth medium in a 50-mL conical tube. Thaw the frozen cryovial of cells within 1–2 minutes by gentle agitation in a 37°C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% (v/v) ethanol.
2. Transfer the thawed cell suspension to the conical tube containing 10 ml of growth medium.
3. Collect the cells by centrifugation at 1000 rpm for 5 minutes at room temperature. Remove the growth medium by aspiration.
4. Resuspend the cells in the conical tube in 15 mL of fresh growth medium by gently pipetting up and down.
5. Transfer the 15 mL of cell suspension to a T-75 tissue culture flask. Place the cells in a 37°C incubator at 5% CO2.
6. Monitor cell density daily. Cells should be passaged when the culture reaches 95% confluence.

Recent Product Citations

1. Hashimoto, H. et al. (2016). Study on AAV-mediated gene therapy for diabetes in humanized liver mouse to predict efficacy in humans. *Biochem Biophys Res Commun*. doi:10.1016/j.bbrc.2016.08.104.
2. Lee, S. E. et al. (2016). nArgBP2 regulates excitatory synapse formation by controlling dendritic spine morphology. *Proc Natl Acad Sci U S A.* doi:10.1073/pnas.1600944113.
3. Lutz, D. et al. (2014). Myelin basic protein cleaves cell adhesion molecule L1 and promotes neuritogenesis and cell survival. *J Biol Chem*. **289**:13503-13518.

Warranty

This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.

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