Technical Bulletin

VirusExpress® 293 AAV Production Cells

Catalog Number VP002

Product Description

VirusExpress 293 AAV Production Cells are a clonal, suspension-adapted HEK293 derivative selected for fast growth, high transfection efficiency, and high-titer AAV production. VirusExpress 293 AAV Production Cells grow to high cell densities and are amenable to chemical-based transfection reagents commonly used in manufacturing (i.e., PEI, lipid-nanoparticle). A medium exchange step is not needed prior to transfection, and the cells can be transfected in larger volumes, enabling scale-up of AAV production. The cells are supplied in a chemically defined media formulation and can be thawed directly into EX-CELL® CD HEK293 Viral Vector Medium (Catalog Number 14385C).

VirusExpress 293 AAV Production Cells are derived from the HEK293 cell line (ATCC® CRL-1573)through single cell cloning. The parental HEK293 cell line was established by transformation of human embryonic kidney cells with sheared human adenovirus 5 DNA.[1] A 4-kb adenoviral genome fragment is known to have integrated into chromosome 19 and encodes for E1A/E1B proteins.

The clonal derivative eliminates variability from the population while also being adapted to serum-free suspension culture conditions. Suspension culture and chemically defined medium allow scalability into stirred tank bioreactors for yields that can meet commercial needs.

Components

VirusExpress 293 AAV Production Cells are supplied in a vial containing 1 mL of cells at 1×10^7 viable cells/mL.

The cells are banked under cGMP conditions in 92% chemically defined medium and 8% DMSO. The master and working cell banks have been comprehensively tested for adventitious agents and cell identity.

Precautions and Disclaimer

For Research or Further Manufacturing Uses Only-Not Intended for Direct Use in Humans or Animals. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

A commercial license must be taken prior to use of the cells, or any products or materials derived from the cells, in a manufacturing process.

Please contact your local sales representative for more details.

Storage/Stability

Store the cells at −196 °C (liquid nitrogen) immediately upon arrival.

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Procedure

Stock Culture Initiation/Thawing of VirusExpress® 293 AAV Production Cells

Reagents and Equipment Required but Not Provided

- Sterile Erlenmeyer 125 mL non-baffled cell culture shake flask with vented cap
- Sterile pipettes
- EX-CELL CD HEK293 Viral Vector Medium (Catalog Number 14385C) with addition of 6 mM L-Glutamine (Catalog Number 59202C)
- Water bath at 37 °C
- Type II biosafety cabinet
- Humidified CO_2 incubator (8% CO_2, 37 °C)
- Cell counter
- Orbital shaker plate
- 70% isopropanol

Thawing procedure

1. Warm growth medium (EX-CELL CD HEK ViralVector Medium supplemented with 6 mM L-glutamine) to 37 °C.
2. Thaw vial of cells in a 37 °C temperature bath until just thawed (~2-3 minutes). **Keep cap out of the water in order to avoid contamination**.
3. Spray the vial thoroughly with 70% isopropyl alcohol and wipe down completely prior to transfer into a biosafety cabinet.
4. Gently transfer entire contents of the vial to 20 mL of pre-warmed growth medium in a sterile 125 mL non-baffled shake flask with vented cap.
5. Optional step: Obtain a cell count (e.g., via hemocytometer or automated cell counter).
6. Transfer the shake flask into a shaking, humidified incubator set to 37 °C, 8% CO_2, and 135 rpm (25 mm orbital diameter).
7. Begin Sub-Culturing Procedure after 3 days post-thaw. Viability should exceed 80%.

Sub-Culturing of Cells

Reagents and Equipment Required but Not Provided

- Sterile baffled shake flasks (see Tables 1 and 2)
- EX-CELL CD HEK293 Viral Vector Medium (Catalog Number 14385C) with addition of 6 mM L-Glutamine (Catalog Number 59202C)
- Orbital shaker (see Tables 1 and 2)
- Humidified CO_2 incubator (8% CO_2, 37 °C)
- Sterile pipettes
- Type II biosafety cabinet
- Cell counter
- 70% isopropanol

Sub-Culturing Procedure

1. Passage cells at 6 × 10^5 viable cells per mL for either a 3-day passage or a 2-day passage. **Do not** allow cells to reach viable cell density (VCD) greater than 8 × 10^6 viable cells/mL for optimal performance.
2. Subculture cells for at least three passages prior to transfections.
3. Tables 1 and 2 show the recommended shake flask vessels and respective working volumes of cells for seed train expansion.

Table 1
Parameters for 25 mm Incubator Orbital Diameter

Vessel Size	Working Volume (mL)	Incubator Orbital Diameter (mm)	Shaking Speed (rpm)
125 mL	30	25	135
250 mL	60	25	135
500 mL	120	25	135
1,000 mL	300	25	135
2,000 mL	600	25	135
3,000 mL	1,200	25	96

Table 2
Parameters for 50 mm Incubator Orbital Diameter

Vessel Size	Working Volume (mL)	Incubator Orbital Diameter (mm)	Shaking Speed (rpm)
125 mL	30	50	96
250 mL	60	50	96
500 mL	120	50	96
1,000 mL	300	50	96
2,000 mL	600	50	96
3,000 mL	1,200	50	68

Results

Figure 1
Viable cell density and doubling time. VirusExpress 293 AAV Production Cells were sub-cultured every three to four days up to passage 24. An average doubling time of 29 hours was observed over multiple passages.

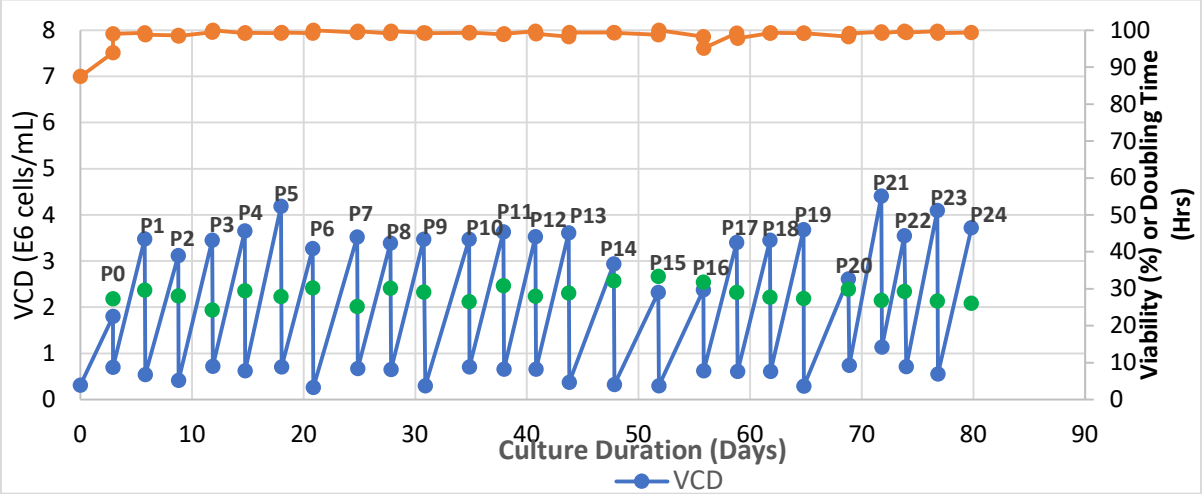


Figure 2.
Growth in Bench-Scale Bioreactors VirusExpress. 293 AAV Production Cells were cultured for batch growth in Mobius® 3 L single-use bioreactors. Cells were seeded in the 3 L bioreactor at 6×10^5 viable cells (vc) per mL. Peak viable cell density (VCD) of 12×10^6 vc/mL was obtained with viability >90%.

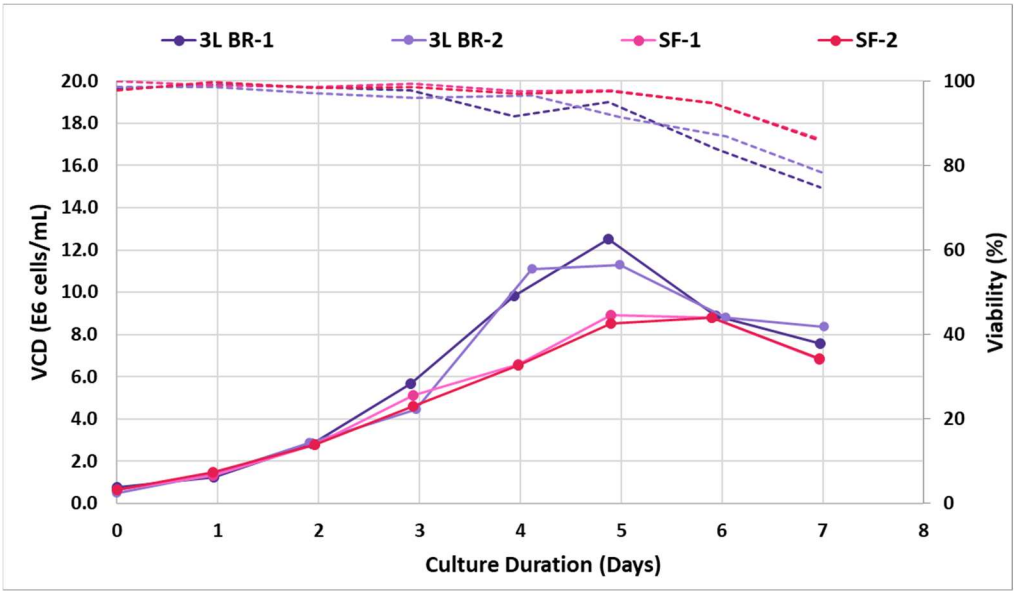
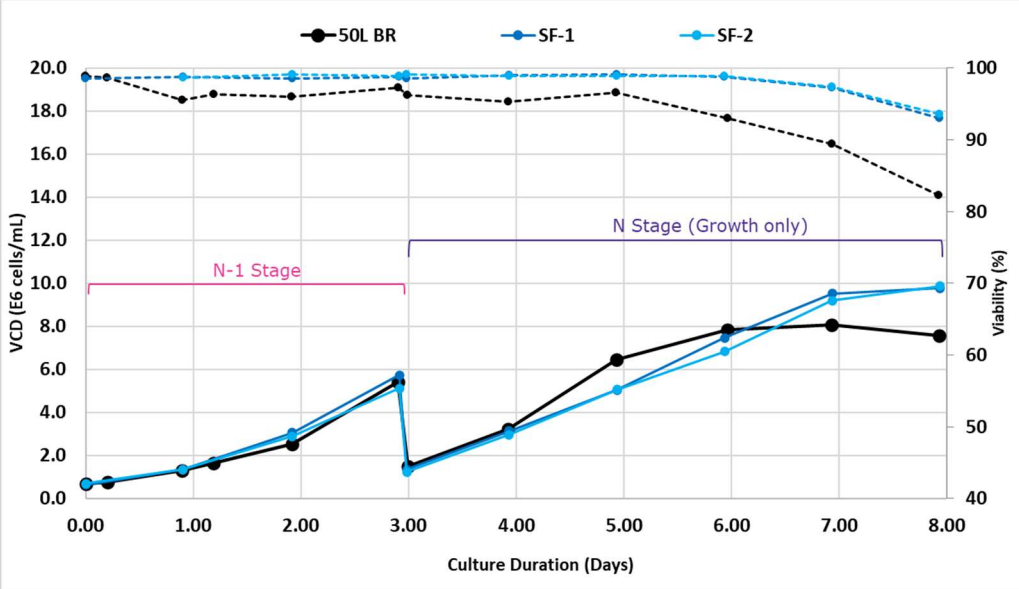


Figure 3
Growth in Large-Scale Bioreactor. VirusExpress 293 AAV Production Cells were cultured for batch growth in Mobius® 50 L single-use bioreactors. Cells were seeded at 6 × 10^5 vc/mL and diluted with fresh media on Day 3. Peak viable cell density (VCD) of 8 × 10^6 vc/mL was obtained with viability >90% in Mobius 50 L bioreactor. Comparable growth of cells was achieved with both bench-scale and large-scale bioreactors.



Frequently Asked Questions

1. **How are VirusExpress 293 AAV Production Cells banked?**
   VirusExpress 293 AAV Production Cells are banked and tested according to current good manufacturing practices (cGMP).

2. **Are the EX-CELL CD HEK293 Viral Vector Medium and the VirusExpress 293 AAV Production Cells serum-free?**
   Yes, both are serum-free.

3. **What is the recommended carbon dioxide (CO_2) level needed to grow VirusExpress 293 AAV Production Cells?**
   We recommend growing the cells at 8% CO_2

4. **Can antibiotics be used in the culturing of VirusExpress 293 AAV Production cells?**
   We do not recommend using penicillin, streptomycin, or hygromycin when culturing VirusExpress 293 AAV Production Cells, because the cells may suffer from a decrease in viability. Other antibiotics may be suitable for selection and induction purposes, such as puromycin, blasticidin, Zeocin®, or doxycycline.

5. **What shake flasks do you recommended using for VirusExpress 293 AAV Production Cells?**
   We recommend using a non-baffled shake flask for cell thawing and a baffled shake flask for subsequent suspension culture to reduce cell clumping at 135 rpm (25 mm orbital diameter).

6. **How should I count the VirusExpress 293 AAV Production Cells?**
   Inaccurate cell counting methods can lead to inappropriate seeding density and poor cell growth. Make sure counting samples are adequately mixed to avoid local variations in cell density that can affect the accuracy of cell counts. Recheck all calculations used to determine counts using a hemocytometer or consider automated cell counting.

7. **How I can improve accuracy of VirusExpress 293 AAV Production Cells counts?**
   Because VirusExpress 293 AAV Production Cells may grow in clusters of 2–10 cells, collect a 600 µL cell sample, add an equal volume of Accumax™ solution (Catalog Number A7089), and mix gently. Incubate tubes at 37 °C on a shaker platform for 30 minutes. Pipette the samples 5–6 times to break up remaining cell aggregates and transfer the volume required for automated cell counter measurement.

8. **How should a DNA co-transfection using several DNA plasmids be performed?**
   For co-transfection of multiple plasmids, we recommend the total DNA amount does not exceed the maximal DNA amount indicated in the transfection reagent manufacturer's protocol.

9. **What is the suggested cell density of VirusExpress 293 AAV Production Cells for routine culture in EX-CELL CD HEK293 Viral Vector Medium?**
   We recommend inoculating cell cultures at 6×10^5 viable cells/mL for either 2-day or 3-day cultures.

10. **What cell density should I seed VirusExpress 293 AAV Production Cells 24 hours before transient PEI transfection?**
    While viable cell densities at the time of transfection can be optimized to improve viral titers, a good starting point is to seed 1.1×10^6 viable cells/mL 18-24 hours before transient transfection. A good, targeted cell density on the day of transfection is $2.0\text{-}2.5 \times 10^6$ viable cells/mL with a viability >95%. If the split ratio of cells to fresh medium is less than 1:2, we recommend spinning down the cell suspension and resuspending the cell pellet in fresh, pre-warmed EX-CELL CD HEK293 Viral Vector Medium.

11. **What transfer plasmid to packaging plasmids ratio should I use?**
    All plasmid ratios need to be optimized for each transfection to obtain the best AAV titers. Please note the quality of plasmids, the ratio of the plasmids, and the vector design will all contribute to the titer. We recommend trying vector ratios ranging from 1:3 to 1:1 of transfer plasmid:packaging plasmids by weight.

12. **What DNA to PEI ratio should I use?**
    The DNA:PEI ratio should be optimized with the other transfection parameters; typically, one might begin with a DNA:PEI ratio of 1:3 or 1:2. Consult the transfection reagent manufacturer's protocol for additional recommendations.

References

1. Graham, F.L., *et al*., Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen. Virol*., **36**, 59-74 (1977).
2. Xiao, X., Li, J. and Samulski, R.J., Production of high-titer recombinant adenoassociated virus vectors in the absence of helper adenovirus. *J. Virol*., **72**, 2224–2232 (1998).
3. Matsushita, T., *et al*., Adeno-associated virus vectors can be efficiently produced without helper virus. *Gene Ther*., **5**, 938–945 (1998).
4. Durocher, Y., *et al*., Scalable serum-free production of recombinant adeno-associated virus type 2 by transfection of 293 suspension cells. *J. Virol. Methods*, **144**, 32–40 (2007).
5. Hildinger, M., *et al*., High-titer, serum-free production of adeno-associated virus vectors by polyethyleneimine-mediated plasmid transfection in mammalian suspension cells. *Biotechnol. Lett*., **29**, 1713-1721 (2007).
6. Park, J.Y., *et al*., Scalable production of adeno-associated virus type 2 vectors via suspension transfection. *Biotechnol. Bioeng*., **94**, 416-430 (2006).

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