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Product Information

VirusExpress™ 293T Lentiviral Production Cells

Catalog Number **VP001**
Storage Temperature –196 °C (liquid nitrogen)

TECHNICAL BULLETIN

Product Description

VirusExpress™ 293T Lentiviral Production Cells are a clonal, suspension-adapted HEK293T derivative selected for fast growth, high transfection efficiency, and high-titer lentivirus production. VirusExpress 293T Lentiviral Production Cells grow to high cell densities, exhibit minimal cell clumping, and are amenable to PEI-based transfection. A medium exchange step is not needed prior to transfection with a PEI transfection reagent, and the cells can be transfected in larger volumes, enabling scale-up of lentivirus production. The cells are supplied in a chemically defined media formulation and can be directly thawed into EX-CELL® CD HEK293 Viral Vector Medium (Catalog Number 14385C).

VirusExpress 293T Lentiviral Production Cells are derived from the HEK293T cell line (ATCC® CRL-3216) 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.

HEK293T cells were created by stable transfection of the HEK293 cell line with a temperature-sensitive mutant of SV40 large T antigen. Due to the expression of the large T antigen, transfected plasmids that carry the SV40 origin of replication can replicate in 293T cells and will transiently maintain a high copy number. The clonal derivative eliminates variability from the population while also being adapted to suspension, serum-free culture conditions. Suspension culture and chemically defined medium allow scalability into stirred tank bioreactors for yields that can meet commercial needs.

Components

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

The cells were banked under cGMP conditions in 92% chemically defined medium supplemented with glutamine 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.

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.

Do not store at −80 °C.

Procedures

Stock Culture Initiation/Thawing of VirusExpress 293T Lentiviral Production Cells

Reagents and Equipment Required but Not Provided.

- Sterile Erlenmeyer 125 mL baffled cell culture flask with vented cap
- 15 mL sterile conical tube
- 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
- Centrifuge capable of spinning 15 mL conical tubes
- 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 Viral Vector Medium supplemented with 6 mM L-glutamine) to 37 °C.
2. Thaw vial of cells in a 37 °C temperature bath until just thawed. 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 9 mL of pre-warmed growth medium and spin cells down for 5 minutes at 18–23 °C at 200 × *g*.
5. Decant about 9.5 mL of supernatant and leave ~0.5 mL. Gently flick the bottom of the centrifuge tube to resuspend the cells.
6. Add 5 mL of growth medium and transfer to 25 mL of growth medium in a sterile 125 mL baffled shaker flask with vented cap.
7. Obtain a cell count prior to transfer of the shaker flask into a shaking, humidified incubator set to 37 °C, 8% CO_2, and 135 rpm (25 mm orbital diameter).

Sub-Culturing of Cells

Reagents and Equipment Required but Not Provided.

- Sterile 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. Subculture cells on day 3 from the thaw. Viability should exceed 80%.
2. Passage cells at 3×10^5 viable cells/mL for a 4-day passage or at 5×10^5 viable cells/mL for a 3-day passage. Do not allow cells to reach viable cell density (VCD) greater than 8×10^6 viable cells/mL for optimal performance. Discard cells after 30 passages.
3. Subculture cells for at least three passages prior to transfections.
4. Tables 1 and 2 show the recommended shaker flask vessels and respective working volumes of cells for seed train expansion.

Table 1.
Parameters for 25 mm Incubator Orbital Diameter

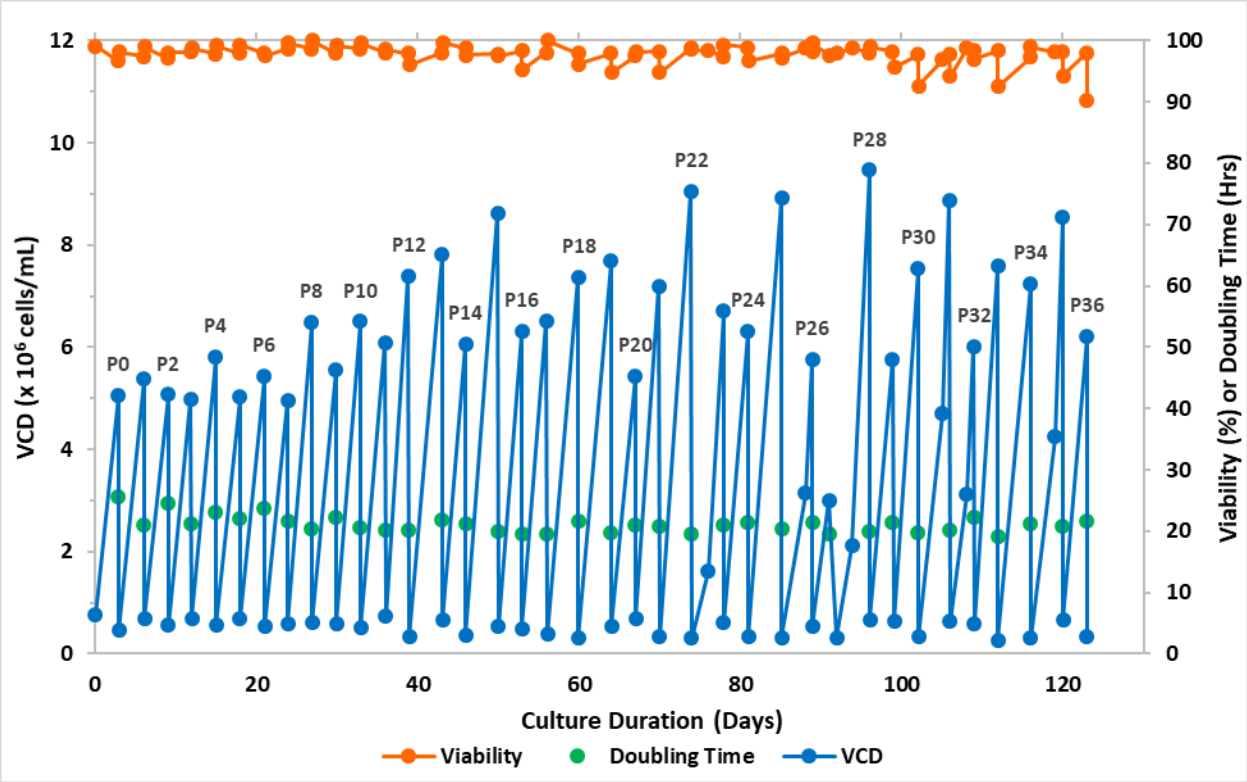
Vessel Size	Working Volume (mL)	Incubator Orbital Diameter (mm)	Shaking Speed (rpm)
125 mL	30	25	135
250 mL	60	25	135
500 mL	120	25	135
1,000 mL	300	25	135
2,000 mL	600	25	135
3,000 mL	1,200	25	96

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

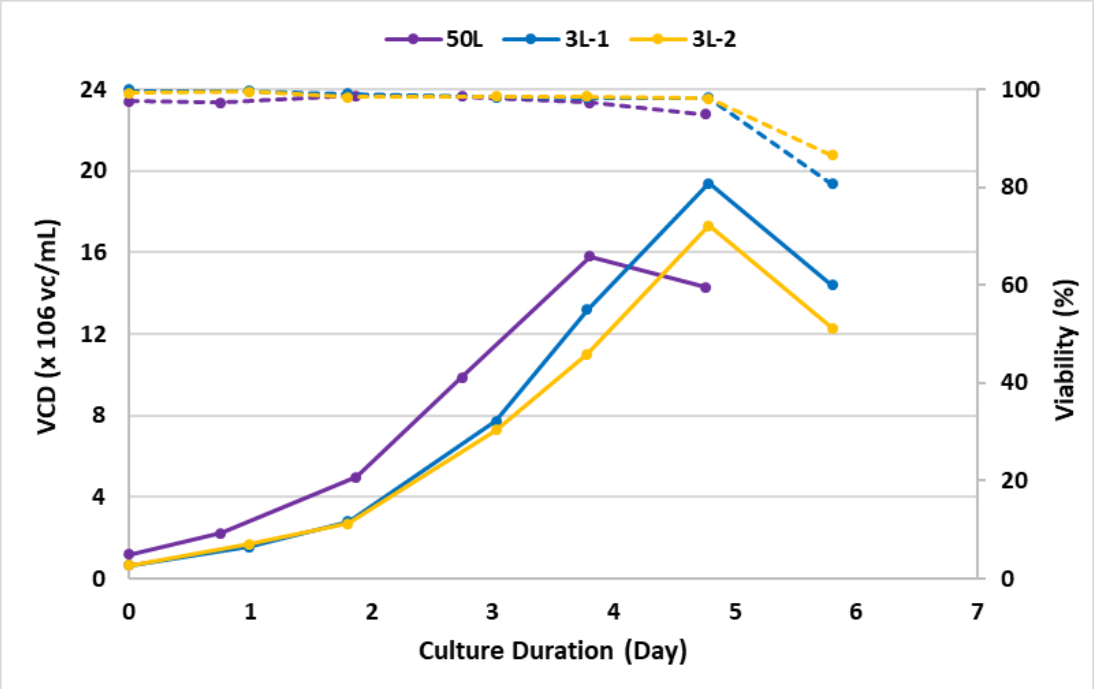
Results

Figure 1.
Viable cell density and doubling time



VirusExpress 293T Lentiviral Production Cells were sub-cultured every three to four days up to passage 36. An average doubling time of 21 hours was observed over multiple passages.

Figure 2.
Growth in Bioreactors



VirusExpress 293T Lentiviral Production Cells were cultured for batch growth in Mobius® 3 L and 50 L Single-Use Bioreactors. While cells were seeded in the 50 L bioreactor at 1×10^6 viable cells/mL (vc/mL) and in the 3 L bioreactor at 5×10^5 vc/mL, comparable growth of cells was achieved at both scales. Peak viable cell density (VCD) of 16–20 $\times$ 10^6 vc/mL was obtained with viability >95%.

Frequently Asked Questions

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

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

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

4. **Can antibiotics be used in the culturing of VirusExpress 293T Lentiviral Production cells?**
   We do not recommend using penicillin, streptomycin, or hygromycin when culturing VirusExpress 293T Lentiviral Production Cells, because they may suffer from a decrease in viability. Also avoid G418 (Geneticin®), since HEK293T cells have previously been selected using neomycin for the large T antigen. Other antibiotics may be suitable for selection and induction purposes, such as puromycin, blasticidin, Zeocin®, or doxycycline.

5. **What shaker flasks do you recommended using for VirusExpress 293T Lentiviral Production Cells?**
   We recommend using a baffled shaker flask for suspension culture to reduce cell clumping at 135 rpm (25 mm orbital diameter).

6. **How should I count the VirusExpress 293T Lentiviral 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 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.

8. **How I can improve accuracy of VirusExpress 293T Lentiviral Production Cells counts?**
   Because VirusExpress 293T Lentiviral 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.

9. **What is the suggested cell density of VirusExpress 293T Lentiviral Production Cells for routine culture in EX-CELL CD HEK293 Viral Vector Medium?**
   We recommend inoculating cell cultures at 5×10^5 viable cells/mL for 3-day cultures or 3×10^5 viable cells/mL for 4-day cultures.

10. **What cell density should I seed VirusExpress 293T Lentiviral Production Cells 24 hours before transient PEI transfection?**
    While viable cell densities at the time of transfection can be optimized in order to improve viral titers, a good starting point is to seed 1.2×10^6 viable cells/mL 18–24 hours before transient transfection. A good targeted cell density on the day of transfection is $2.2–2.7 \times 10^6$ 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 in order to obtain the best functional lentivirus titers. Please note the quality of plasmids, the ratio of the plasmids, and the vector design will all contribute to the functional viral 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. 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. *Journal of General Virology*, **36:1**, 59-74 (1977).
2. Dull, T., *et al.,* A Third Generation Lentivirus Vector with a Conditional Packaging System. *J. Virol*., **72(11)**, 8463–8471 (1998).
3. Naldini, L., *et al.,* In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science*, Apr 12, **272(5259)**, 263-7 (1996).
4. Miyoshi, H., *et al*., Development of a self-inactivating lentivirus vector. *J. Virol*., Oct, **72(10)**, 8150-7 (1998).

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