ELEVECTA™ transient cell line

GENE THERAPY TECHNOLOGY

The ELEVECTA™ transient cell line is designed for speed. Our off-the-shelf transient cell line is crafted with simplicity in mind, making it easy for researchers and technicians to integrate it into their existing workflows.

The ELEVECTA transient cell line is a flexible option for producing viral vectors using any transient transfection method. To achieve the desired productivity, Cytiva also offers HyClone™ prime expression medium, which is optimized specifically for the ELEVECTA transient cell line. It's designed to reduce development time and quickly advance promising candidates to clinical trials. Our transient cell line is the foundation of any gene therapy research campaign.

As part of the ELEVECTA cell line portfolio, the transient cell line provides speed at the early development stage with the potential to transition to our packaging and producer cell lines. This overall portfolio is designed to meet diverse therapy needs and provide a competitive edge in product development.

ELEVECTA transient cell line offers the following benefits:

- **Excellent performance:** Achieve high titers of infectious virus, which can be boosted further by using enhancers.
- **Enhanced product quality:** Minimize host cell DNA (hcDNA) inside capsids, which is resistant to nucleases and chromatographic clearance.
- **Innovative:** Access advances in cell line engineering across the entire cell line portfolio to support transition between cell lines as your needs evolve.
- **Collaborative:** We're more than a supplier. Benefit from a comprehensive suite of regulatory support services designed to expedite your success in advancing to and through clinical trials.

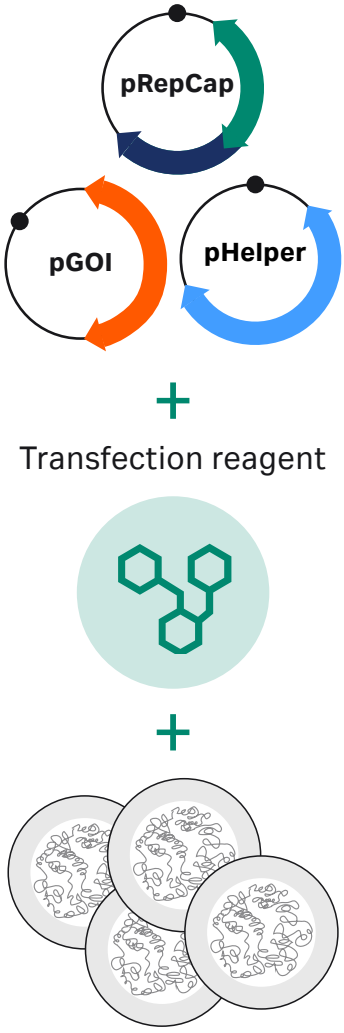

Fig 1. Transient transfection process.

Platformability

The ELEVECTA transient cell line, grown in HyClone prime expression medium, demonstrates robust performance across various serotypes. Addition of the RevIT enhancer from MirusBio further amplifies titers by a factor of 2 to 3, underscoring the cell line's ability to further increase its performance.

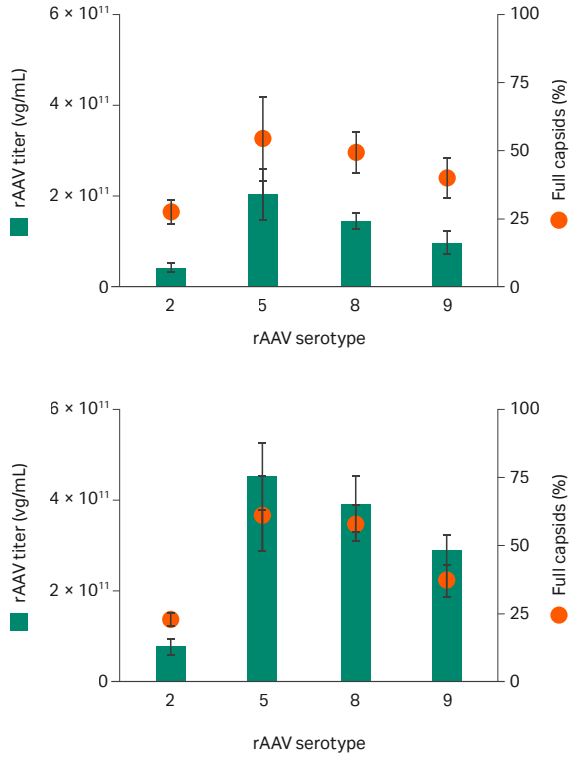

Fig 2. Recombinant adeno-associated virus (rAAV) production performance in ambr 15 bioreactor. rAAV titers were analyzed by quantitative PCR (qPCR)/ELISA and used for calculation of packaging efficiencies. Cells were transfected using PEI MAX and a standard 3-plasmid system. (Top) Non-enhanced ambr15 bioreactor baseline process (n = 6). (Bottom) Enhanced (RevIT AAV enhancer) ambr15 bioreactor process (n = 6).

Scalability

Ensuring scalability within a gene therapy program is paramount, and the ELEVECTA transient cell line has proven its performance across varying scales, including 3 L and 10 L stirred tank bioreactors. This cell line consistently maintains high viral titers and capsid fullness levels, demonstrating reliability and efficacy irrespective of the bioreactor size.

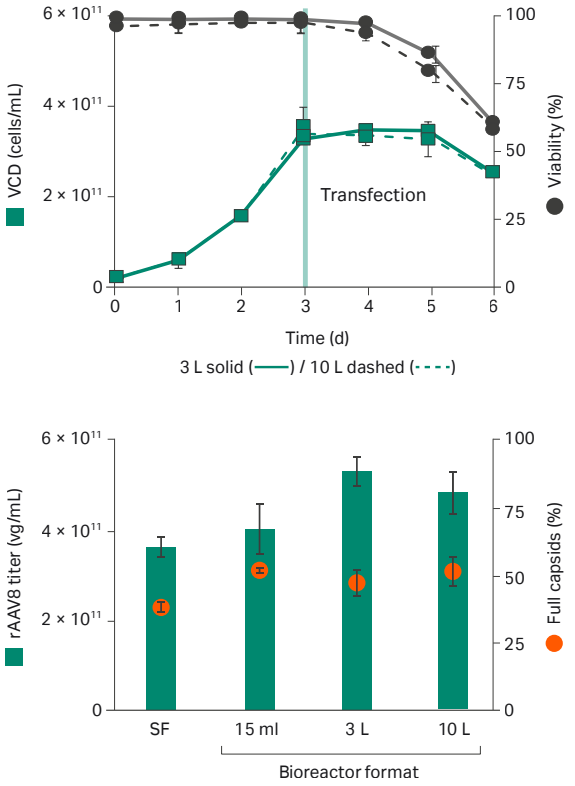

Fig 3. Bioreactor scale-up for rAAV8 and process comparability. (Top) Viable cell density (VCD) and viability of the ELEVECTA transient cell line in 3 L (n = 3) and 10 L (n = 3) bioreactors in presence of RevIT AAV enhancer. Transfection and supplementation was performed 3 days post-seeding. (Above) Comparison of rAAV8 genomic titers and fullness in shake flasks (SF, n = 2), ambr15 (n = 6), Applikon 3 L or Xcellerex™ XDR-10 L bioreactors (each n = 3). rAAV titers were analyzed by droplet digital PCR (ddPCR)/ELISA and used for calculation of packaging efficiencies.

Enhanced quality

The ELEVECTA transient cell line exhibits a notable decrease in encapsidated hcDNA within the final viral vector product. The presence of encapsidated hcDNA holds significant implications for the regulatory approval process of gene therapy. With the ELEVECTA transient cell line we demonstrate ≤ 12.4 nanograms (ng) hcDNA per 10^{14} vg in the 10 L scaled-up process, a 100-fold reduction over another commercially available cell line. This level of hcDNA reduction can substantially simplify the risk assessments typically needed to justify overall residual DNA levels in rAAV therapies.

ELEVECTA transient cell line carries a knockout of the caspase-activated DNase coding gene DFFB which suppresses apoptosis-mediated DNA fragmentation in the cells and thus significantly reduces the packaging of hcDNA into rAAV particles. In contrast to the unpackaged DNA co-purified with the AAV vector product, encapsidated hcDNA cannot be removed by conventional downstream purification processes. The novel cell line described herein suppresses the undesirable packaging of hcDNA during production.

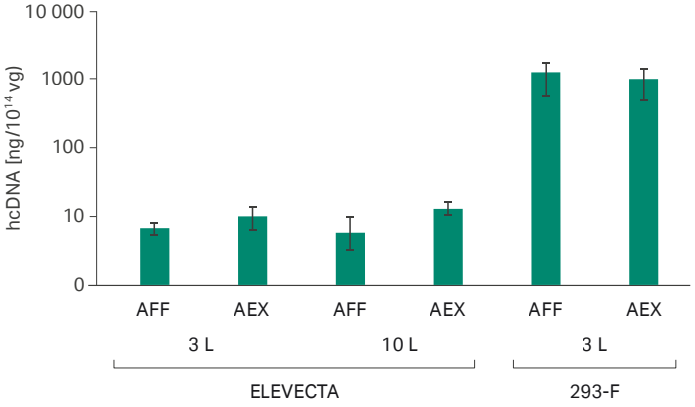

Fig 4. Comparison of hcDNA (including encapsidated) levels (ng per 10^{14} viral genomes) after purification of viral vectors via affinity (AFF) and anion exchange chromatography (AEX) enrichment and analysis with a commercial residual hcDNA qPCR assay (n = 3). ELEVECTA transient cell line and FreeStyle 293-F derived rAAV8 are depicted. Statistical analysis was performed using Welch's t-test, confirming a statistically significant difference for hcDNA as a proportion of viral genome DNA between ELEVECTA and 293-F in all possible comparisons and combinations ($p < 0.05$).

Cell line basic characteristics

ELEVECTA transient cell line:

- Clonal HEK293 cell line derived from the parental HEK293 suspension-adapted cell line from Cytiva.
- Adapted to the HyClone prime expression medium.
- Optimized for high-density suspension culture (> 1 × 10^7 cells/mL).

1. Cell line growth performance

ELEVECTA transient cell line exhibits peak viable cell densities of > 1 × 10^7 cells/mL and an average cell doubling time of 20 h (exponential phase).

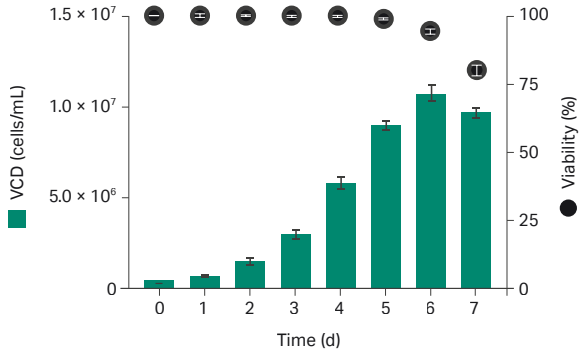

Fig 5. Analysis of growth characteristics of ELEVECTA transient cell line in HyClone prime expression medium under batch culture conditions in shake flask format (n = 3). An initial seeding density of 2 × 10^5 cells/mL was used.

2. Cell line stability

ELEVECTA transient cell line demonstrates exceptional stability in HyClone prime expression medium and exhibits consistent rAAV productivity for multiple serotypes over 70 cell doublings.

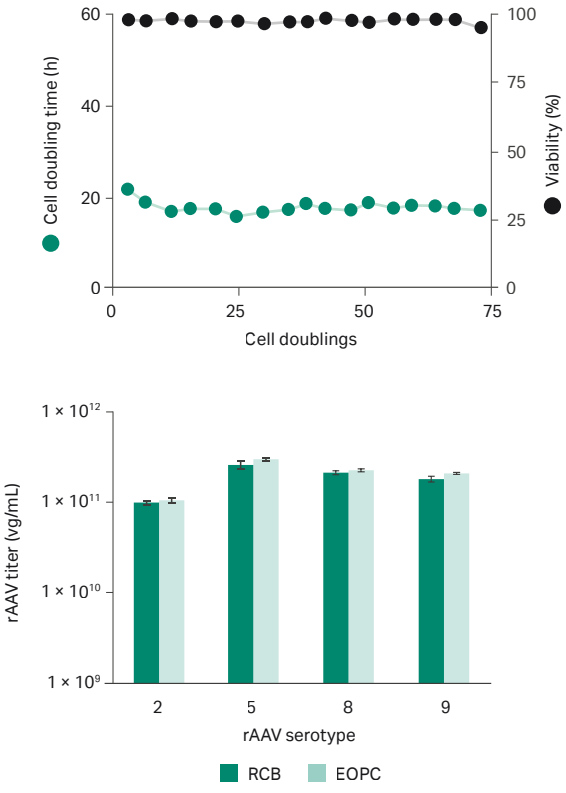

Fig 6. Analysis of cell line stability. (Top) Growth characteristics of ELEVECTA transient cell line for up to 70 cell doublings. (Bottom) Comparison of rAAV production performance of RCB (research cell bank) and EOPC (end of production cell line), 70 cell oublings for serotypes 2, 5, 8, and 9 (n = 3). rAAV titers were analyzed by qPCR.

Transitioning to stable cell lines

The ELEVECTA™ transient cell line is part of the ELEVECTA cell line portfolio which also includes packaging and producer cell line options. This allows to transition between different cell lines as needs evolve. Interconnected through their common parental cell line, the three cell lines create a complete portfolio, supporting regulatory and quality uniformity.

Regulatory support

The ELEVECTA transient cell line benefits from the comprehensive support of our regulatory and technical teams, underpinned by an unwavering commitment to quality.
The ELEVECTA transient cell line is available in research grade and good manufacturing practices (GMP) grade. The GMP grade is manufactured in accordance with EU current GMP standards (Eudralex Volume 4) and characterized as per ICH Q5A and ICH Q5D.

Specifications

Cell format	Frozen
Cell type	Kidney; embryo
Growth properties	Suspension
Cell concentration per vial	1.5×10^7 cells
Vial volume	1.8 mL
Storage conditions	Vapor phase of liquid nitrogen
Media of suspension	HyClone prime expression medium

Ordering information

Product	Pack size	Product code
ELEVECTA transient cell line – Research Only	1 Vial	ETCL-RUO
ELEVECTA transient cell line – GMP	1 Vial	ETCL-GMP
HyClone prime expression medium, dry powder (please contact directly your Cytiva representative for ordering)	5 L	SH31198.01
	10 L	SH31198.02
	50 L	SH31198.03
	100 L	SH31198.04
	500 L	SH31198.05
	1000 L	SH31198.06
HyClone prime expression medium, liquid, bags (please contact directly your Cytiva representative for ordering)	1 L	SH31199.01
	1L (bottle)	SH31199.02
	5 L	SH31199.03
	100 L	SH31199.04
	200 L	SH31199.05
	10 L	SH31199.06
	20 L	SH31199.07
	50 L	SH31199.15

If you have any questions regarding our cell line products, please contact your local sales representative or email us at ELEVECTA@cytiva.com.

You can also request a quote online at https://cytiva.link/producer-DF

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