Lentiviral Vector Lab-Scale Production

From Research to the Clinic

Simplifying Progress

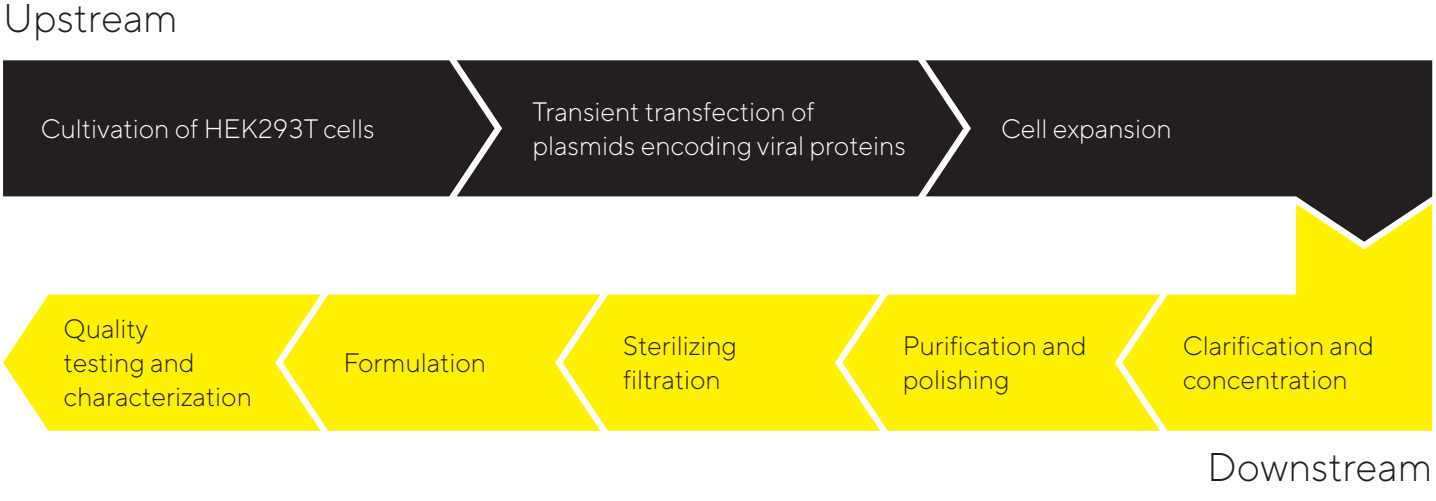
SARTORIUS


3000 mL
2500
2000
1500
1000
500
250 mL
(Approx.)
SARTORIUS
4Cell®
BHK-21 CD Medium
Chemically Defined
5L Flexsafe® Bag
Order no.: CFF3FA3110
Lot number: XXXX
Volume: 5 L
Mfg. date: 03.2021
Store at: 2-8°C
For Research or Further Manufacturing Use
Manufactured in Switzerland for Sartorius Stedim Biotech GmbH, 37079 Goettingen, Germany
SARTORIUS
4Cell®
MDCK CD Medium
Chemically defined
1 L Bottle
Order no.: CFV3FA2000
Lot number: 191227A01
Volume: 1000 ml
Mfg. date: 12.2019
Store at: 2-8°C
For Research Use Only
CFV3FA2000
Manufactured in China for Sartorius Stedim Biotech GmbH 37079 Goettingen, Germany
SARTORIUS
4Cell® XtraCHO
Stock & Adaptation Medium
Chemically Defined
1L Bottle
Order no.: CFP3FA1103
Lot number: NDS12011B
Volume: 1000 ml
Exp. date: 03.2021
Store at: 2-8°C
For Research or Further Manufacturing Use
Manufactured in Switzerland for Sartorius Stedim Biotech GmbH, 37079 Goettingen, Germany
SARTORIUS
4Cell® XtraCHO
Feed Medium B
Chemically defined
125 mL Bottle
Order no.: CQP3FA4109
Lot number: XXXX
Volume: 125 ml
Exp. date: 03.2021
Store at: 2-8°C
For Research or Further Manufacturing Use
Manufactured in Switzerland for Sartorius Stedim Biotech GmbH 37079 Goettingen, Germany
SARTORIUS
4Cell® Buffer
WFI
Chemically defined
1 L Bottle
Order no.: CFV3FA1000
Lot number: XXXX
Volume: 1000 ml
Mfg. date: 04.2020
Store at: 2-8°C
For Research or further Manufacturing use
Manufactured in US for Sartorius Stedim Biotech GmbH 37079 Goettingen, Germany

Lentiviral Vector Lab-Scale Production: From Research to the Clinic

Lentiviruses are RNA viruses of the retrovirus family. Their use in the clinical setting has increased in recent years due to their ability to integrate transgenes into both dividing and non-dividing cells, making them highly efficient in gene delivery. In cell-based therapies, especially in chimeric antigen receptor (CAR) T-cell therapy, the use of lentiviral vectors is the method of choice as they offer high efficiency, stability and versatility of gene transfer.

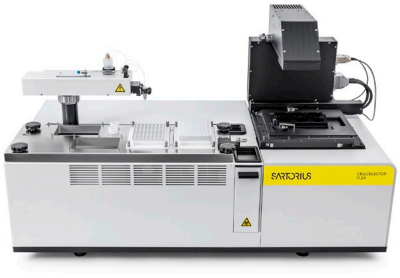
The production of lentiviral vectors centers around the use of HEK293T cell line to produce the viral vector particles. The goal is to achieve high virus yields while maintaining high quality, stability and purity of the virus particles. The entire production workflow may be split into upstream and down-stream processes as illustrated in the below flowchart:



Upstream Production

The production of lentiviral vectors begins with the growth of a cell line, such as derivatives of HEK293. Cells are expanded and transiently transfected with plasmids of DNA encoding the viral proteins. The use of transient transfection is usually preferred over stable producing cell lines as it offers high flexibility. Also, some lentiviral vector components are known to be cytotoxic and therefore may not be ideal for stable cell line culture. These steps may be optimized for titers using various cell media, transfection reagents and culture conditions. The vectors produced are subsequently clarified and purified in downstream steps.

Culture Media



CellCelector Automated Cell Selection and Retrieval Platform

Ambr® 15 Cell Culture Bioreactor System

Cultivation of HEK293 Cells

HEK293 is the predominant cell line used in viral vector production as they are highly amenable to transfection and are known to be very reproducible.

- Four HEK 293 culture media are available: HEK ViP NX, HEK ViP NB, HEK TF and HEK GM, each with a different composition for specific nutritional requirements
  - For all types of viral vector production
  - Proven performance with well-known cells
  - Compatible with most known transfection reagents

Single Cell Selection

CellCelector Automated Cell Selection and Retrieval Platform for:

- Single cell cloning and cell line development for lentivirus packaging and producing cell lines
- Automated selection and isolation of bacterial clones during pDNA production prior to lentiviral vector production
- Detection, selection and isolation of single cells, clusters, spheroids and organoids with:
  - Complete documentation proving monoclonality
  - Improved cell viability during cell cloning and expansion
  - Accelerated workflows and reduced hands-on time by replacing manual steps with automated solution

Small to Large Scale Cell Culture Optimization

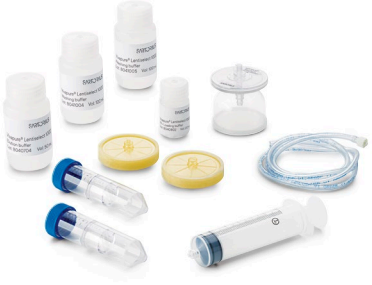
The micro-scale bioreactor system Ambr® 15 Cell Culture mimics the features and processes of large-scale bioreactors.

- High-throughput parallel processing allows for systematic investigation of critical process parameters such as stirring speed, culture pH and transfection steps to assess the impact on lentivirus titer yield and enable process optimization.
- Predicts performance outcome more accurately than traditional shake flasks
- Save on media costs running at 10 – 15 mL working volumes
- Investigate many conditions with 24 or 48 bioreactors operated in parallel
- Embedded MODDE® DOE supports large QbD studies
- Data connectivity to Octet® BLI facilitates transfer of titer analysis data from bioreactor samples back to the Ambr® software

Downstream Production

Lentiviral vectors produced in the upstream process is harvested from the culture media supernatant via clarification, purification, concentration, and a final sterile filtration step. During these steps, cells and cell debris are removed and the viral vectors subsequently purified and concentrated before finally sterile filtered for fill and finish.

Sartoclear Dynamics® Lab



Vivapure® LentiSELECT kit

Minisart® Syringe Filters

Lab-Scale Harvest and Clarification of Lentiviral Vectors

The removal of cells and debris is the first downstream processing step after lentiviral vectors are produced.

- The Sartoclear Dynamics® Lab series of diatomaceous earth and Sartolab® vacuum filtration units simplifies harvest and clarification of cell cultures with a one-step membrane filtration method, reducing processing time
  - Circumvents a centrifugation step
- Filter aid reduces filter clogging issues
- Removes more impurities and reduces turbidity
- Different membrane pore sizes available for different viral vectors

Purification and Concentration

Next, lentiviral vectors are purified to remove impurities.

- Vivapure® LentiSELECT kits enable much faster lentivirus purification when compared to ultracentrifugation:
  - Fast and simple to use
  - High virus purity for increased reproducibility and gene transfer efficiency
- When used in combination with Vivaspin® centrifugal ultrafilters, the purified virus can be concentrated and exchanged into a suitable buffer for storage or further use

Sterilizing Filtration

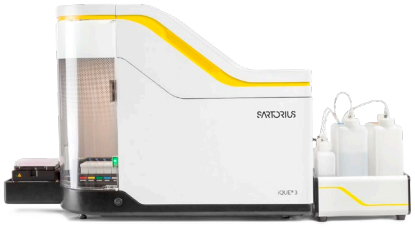
The final step of downstream production is sterile filtration, where adventitious agents such as bacteria or fungi are removed.

- The Minisart® syringe filters keeps the purified viral vectors sterile for subsequent fill and finish

Quality Testing and Characterization

The lentiviral vectors produced must undergo release testing and characterization to ensure safety, efficacy, quality, and lot-to-lot consistency. Critical product and process-related attributes like titer, identity, purity, potency and safety need to be examined. Sartorius provides a comprehensive range of analytical solutions and testing for the assessment of key quality attributes.

Octet® BLI platform



iQue® Advanced Flow Cytometry Platform

Incucyte® Live-Cell Analysis Platform

Titer Analytics

In titer analysis, both total and infectious viral particle numbers are critical quality attributes. However, the infectious viral particle count may be of greater interest as it determines the number of functional viral particles in the test sample.

- For lentiviral particle count, p24 capsid protein-based quantitation assay on the Octet® biolayer interferometry (BLI) platforms may be used.
  - 21 CFR Part 11 compliant
- For infectious particle count, the iQue® Advanced Flow Cytometry Platform may be used to determine the infectious virus titer by flow cytometric measurement of gene expression after viral transduction of cells.
  - Fast plate sampling, integrated analysis, multiplexed no-wash assays
  - Low assay volumes and sample volumes required
  - 21 CFR Part 11 compliant
- Alternatively, the Incucyte® Live-Cell Analysis Platform may be used to determine infectious titer via a rapid, non-invasive, simplified approach using real-time, kinetic readouts and integrated software
- Enables tracking of viable transduced cells over time
- Provides a broad linear detection range
- Assess viral vector stability
- Aids in-process monitoring and optimization of lentiviral vector production
- Supports compliance with 21CFR software module

Microsart® Rapid qPCR Kits

Microsart® @filter and @media

Sterisart® NF System

Microbiological Contamination Control

Rigorous testing for adventitious agents in the lentiviral vector product as well as the materials used in its production is crucial in ensuring patient safety. During the lentiviral vector production process, testing of the plasmids, master and working cell banks, culture media, buffers and viral vector products for the presence of microbes and mycoplasma is performed.

- Rapid sterility and mycoplasma detection:
  - Microsart® rapid kits for the rapid detection of mycoplasma, bacterial and fungal contamination via qPCR
  - Obtain reliable results within 3h
  - Highest specificity by TaqMan™ probes
  - Limit contamination risk with non-infectious bacterial, fungal and mycoplasma validation standards
  - Validated according to EP 5.1.6 and USP <1223> , EP 2.6.7 and USP 63 for sensitivity, specificity, and robustness
- Bioburden determination:
  - Microsart® @filter and @media for membrane filtration bioburden testing
  - Exclusive touch-free membrane system reduces the risk of secondary contamination
  - Easy colony enumeration and picking thanks to the liftable interior lid
  - Meets regulatory requirements USP <61>, <62> and <1231>, Ph.Eur. 2.6.12, JP 4.05
- Sterility testing of clinical supply via growth-based method with the Sterisart® NF System:
  - Compact, closed, ergonomic design
  - Specially constructed needles for safe and easy piercing
  - Guaranteed sterile barrier for aseptic sampling, for rapid microbial testing
  - Fully compliant with USP <71> , Ph. Eur. <2.6.1> and JP <4.06>
  - Sterisart® Easy Configuration Software: Step-by-step guidance through each sterility test programmed
  - 21 CFR Part 11 compliant

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