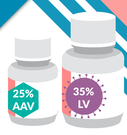
Manufacturing and analytics for lentivirus and AAV vectors: a visual and audio guide

With the successful translation of cell and gene therapies into the clinic, demand for viral vectors has increased significantly. As a result, the need for scalable, cost-effective viral vector manufacturing processes, and improved methods of purification and analytics, has become apparent. Here we show the manufacturing processes and analytics for adeno-associated virus (AAV) and lentivirus (LV) side by side.



Gene-based advanced therapy medicinal products in development

GLOBAL VIRAL VECTOR MANUFACTURING MARKET

2027 US$2.2 billion

2019 US$459.4 million

AAV

AAVs are small non-enveloped viruses containing a linear single-stranded DNA OR self-complementary genome.

FOLLOW THE AAV MANUFACTURING PATHWAY HERE (in green)

LV

Lentiviruses are enveloped RNA viruses containing the reverse transcriptase enzyme that converts RNA into DNA.

FOLLOW THE LV MANUFACTURING PATHWAY HERE (in purple)

Working cell bank

Show me

Show me

UPSTREAM

GENERATION OF MATERIALS NEEDED TO MANUFACTURE THE VIRAL VECTOR

CELL EXPANSION

Cell stock to 200L reactor (or 48 hyperstacks)

3–4 weeks AAV & LV

Cell expansion

Mycoplasma testing can be used for RISK MITIGATION to check your working cell bank and raw materials.

Suspension culture can reduce costs by 50%

Adherent vs suspension culture

Bioreactor

All components used during manufacture, including raw materials and cell and viral banks, must be qualified and suitable for the intended purpose.

ANALYTICS

FOLLOW THE ANALYTICS PATHWAY HERE (in blue)

Show me

Use of serum-free medium during upstream processes reduces the risk of exposing the process to related contaminants and simplifies purification and downstream processing.

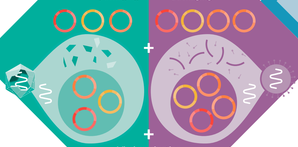
GENERATION OF THE VIRAL VECTOR

Manufacture of viral vectors requires vector backbone components to be combined with the therapeutic gene of interest. This is usually achieved through transient transfection, stably from a vector producer cell line or by infection.

TRANSIENT TRANSFECTION

3 plasmids used for packaging

4 plasmids used for packaging



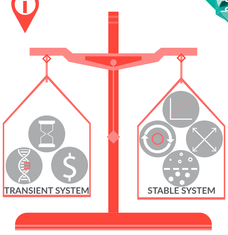
Viral production stimulators

2–4 days

2–4 days

Mycoplasma testing should be performed at cell harvest for lot release.

Transient vs stable systems



Endonuclease

Time of cell harvest should be determined based on both product quality and quantity. Longer culture times lead to lower cell viability and greater purification burden.

DOWNSTREAM

Downstream manufacturing transforms the bulk viral harvest supernatant produced in the upstream process into a product ready for the clinic. The challenge is ensuring regulatory standards for product safety and potency are met whilst maximising yield.

Purification processes must be designed in accordance with virus properties

CLARIFICATION

To remove cells and cell debris
Centrifugation and/or microfiltration

Clarification

No — Yes

ENVELOPE

CONCENTRATION

To reduce volumes
Tangential Flow Filtration
Ultracentrifugation

Concentration

20 nm — 90-120 nm

SIZE

CAPTURE

To remove process-related impurities
Affinity chromatography
Ion exchange or tangential flow filtration

Capture

High — Low

STABILITY

Downstream processes (200L pilot) using transient transfection:

2–4 days

4–8 days

POLISHING

Removal of empty capsids
Ion Exchange Chromatography

Polishing

POLISHING STAGE ANALYTICS
Process development/ characterization analytics:
- Residual plasmid
- Host cell DNA
- Capsid titer
- Vector genome titer
- Analytical centrifugation

LV ONLY:
- Infectious titer

AAV ONLY
- Empty/full capsid ratio analytics

GMP IN-PROCESS ANALYTICS:
- Vector genome titer

FORMULATION

Concentration and buffer exchange into formulation buffer
Tangential Flow Filtration

Diafiltration

DIAFILTRATION STAGE ANALYTICS
- Vector genome titer

TERMINAL FILTER STERILIZATION

Filtration through 0.2µm filter to ensure sterility of final product

Terminal filter sterilization

VIRUS production can account for 40% of the cost of product

FILL AND FINISH

Vector stocks can remain stable for up to 9 years when cryopreserved at –80°C

A nonoptimized process can: Decrease your viral titer by 33% Increase your costs by 40%

RELEASE AND CHARACTERIZATION TESTING

	AAV	Lentivirus
Identity	Capsid/serotype ID Transgene ID	Transgene ID Envelope protein ID (VSV-G)
Strength	Viral genome titer Total viral particles	Infectivity P24 ELISA
Potency	Infectious titer Functional analysis	Infectious titer Insert stability Functional analysis (i.e., protein assay)
Purity	Host cell protein Host cell DNA Residual BSA Residual endonuclease Residual ligand Residual plasmid Residual transfection reagent Residual detergent Genome integrity Protein purity Aggregation Empty/full capsid ratio	Host cell protein Host cell DNA Residual BSA Residual end Residual plasmid Residual transfection reagent Protein purity Aggregation
Compendial assays	Appearance pH Osmolarity	Appearance pH Osmolarity
Safety	Absence of adventitious viruses Absence of replication-competent viruses Sterility Mycoplasma Endotoxin Bioburden	Absence of adventitious viruses Absence of replication-competent viruses Sterility Mycoplasma Endotoxin Bioburden

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