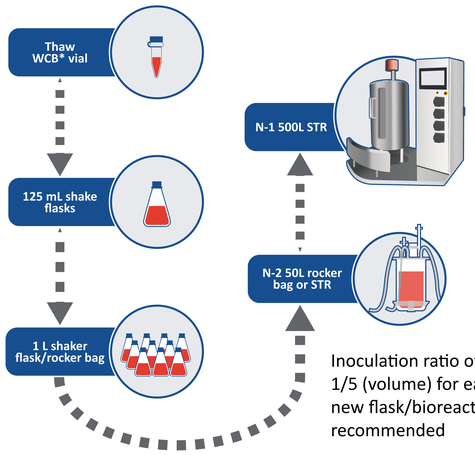
# 2000L scale GMP AAV manufacturing process guidelines using FectoVIR®-AAV

## UPSTREAM PROCESS (Day 0 - 25)

### Seed Train Day 0 - 19




Inoculation ratio of 1/5 (volume) for each new flask/bioreactor recommended

*WCB: Working cell bank

**Tips For Transfection Success:**

- Complexation media should be free of antibiotics & anti-clumping agents.
- Use capacitance for in line VCD assessment for accurate transfection.
- Turn off the pH controller.
- Avoid adding bases as they may decrease transfection efficiency.
- If possible, reduce stir speed and turn off the sparger during complex addition, then back on after.
- Use room temperature solutions to avoid heat/cold shock.

### Transfection Day 19 - 25

Polyplus plasmid engineering services support AAV project


Plasmid DNA (GMP grade – ICH 7)
2-3 plasmid system

Prepare DNA solution by combining plasmid DNA with complexation media.

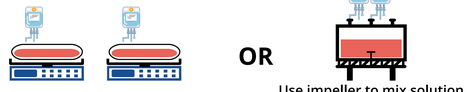

2D bags on rocking platform

100L bag with mixer

Polyplus Transfection Reagent

FectoVIR®-AAV GMP
(mix by inversion before use)

Prepare transfection mix by adding transfection reagent to DNA solution. Do not mix more than 2 mins.




2D bags on rocking platform

Use impeller to mix solution

Add 100 L transfection mix to 2000L bioreactor via gravity or peristaltic pump in a closed system to prevent contamination.

N-stage 2000L production STR

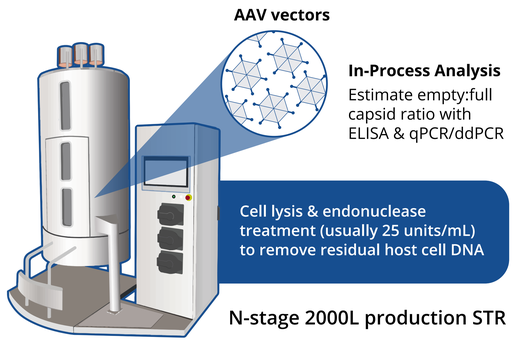
**Key Parameters for Optimization during Process Development:**

1. Validate transfection containers (multiple 2D bags or mixer) & protocol.
2. Define optimal incubation time. Incubation time corresponds to complexation time plus transfer time in bioreactor. Do not exceed this pre-determined specification.
3. Minimize transfer timeframe of transfection mix by taking the largest tubing size available to avoid shearing of complexes. Alternative strategies such as multiple lines can be used.

## DOWNSTREAM PROCESS (Day 25 – 28+)

### Harvest Day 24-26

Collect crude harvest from bioreactor 48h or 72h post-transfection.



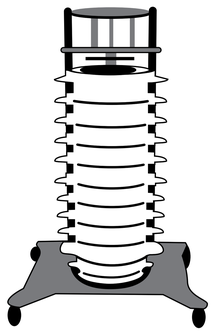

**In-Process Analysis**
Estimate empty:full capsid ratio with ELISA & qPCR/ddPCR

Cell lysis & endonuclease treatment (usually 25 units/mL) to remove residual host cell DNA

N-stage 2000L production STR

### Clarification Day 26-28

Microfiltration or depth filtration to remove contaminations generated during upstream process such as contaminating plasmid DNA, host-cell proteins, host-cell DNA, cell debris.

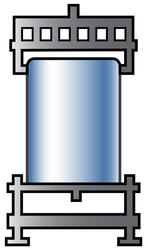


### Purification Day 26-28

Concentrate the viral vectors using ultrafiltration or tangential flow filtration (TFF).

Capture AAV using Affinity Chromatography.

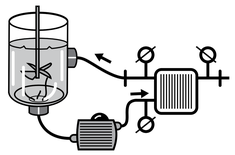
Refine capture using Anion exchange (AEX) Chromatography to remove empty capsids.



### Polishing Day 26-28

Eliminate leachables using TFF.

Filter with single use 0.2 µm sterile filters.



### Fill/Finish Day 28+

**Formulation to the final product concentration**

Manual or automated vial fil of defined dose in inert primary packaging.

Label vials include product name, company, expiry date, lot number, volume/dose, storage conditions, license number, description.

Store product following Good Distribution Practice (GDP) guidelines under conditions identified by stability studies.

### Quality Control/Analytics for Batch Release Day 28+

| Category | Test | Method |
|---|---|---|
| Identity | Appearance | EP 2.2.2 |
| | Osmolality | EP 2.2.35 |
| | Conductivity | USP <644> |
| | pH | EP 2.2.3 / USP <791> |
| | Identity | Product specific |
| | Particulates | ELISA / AUC |
| | Gene expression | Product specific |
| Potency | Transgene function | Product specific |
| | Infectivity | Product specific |
| | Concentration | ELISA |
| | Endotoxin | EP 2.6.14 / USP <85> |
| Safety | Mycoplasma | EP 2.6.7 / USP <63> |
| | BioBurden | EP 2.6.12 / USP <61> |
| | Sterility | EP 2.6.1 / USP <71> |
| | Adventitious agents | ddPCR / ELISA |
| | Endogenous viruses | ddPCR / ELISA |
| | Host cell protein | ELISA / HPLC / SDS-PAGE |
| | Host cell DNA/RNA | ddPCR |
| Purity | Truncated viral protein | ELISA / HPLC / SDS-PAGE |
| | Residual transfection reagent | Commercial assay |
| | Residual helper virus | ddPCR |
| | Residual viral DNA | ddPCR |
| | Leachables | GC/MS |
| | Residual benzonase | Commercial assay |
| | Residual resin ligand | Commercial assay |
| | Heavy metals | USP <232> / <233> |
| | Residual animal-derived components | MS |
| | Detergent | MS |

For in-process testing and batch release, determine the identity, potency, safety & purity of the vector product using validated analytical methods.

Analytical methods to determine quality of AAV particles

| Test | Method |
|---|---|
| Empty/full ratio | SEC-MALS/AUC/TEM/CDMS |
| Replication competent vectors (rc AAV) | Infectivity test |
| Aggregation | SEC / DLS / TEM |
| Glycosylated AAV | SEC / DLS |
| Deamidated AAV | SEC / DLS |

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