

Optimizing AAV Manufacturing at 2,000L Scale: Smart. Scalable. Reliable.

The gene therapy industry continues to expand rapidly, with treatments targeting broader indications and larger patient populations. Therefore the pressure to deliver scalable and cost-effective manufacturing processes is a priority. Adeno-associated virus (AAV) production relying on transient transfection of HEK293 cells remains the trusted and proven strategy to develop these therapies.

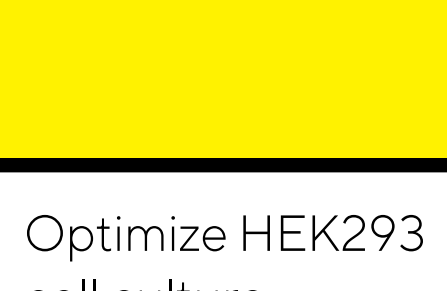
Currently, scale of production ranges from 200 L to 1,000 L scale depending on the clinical stage of the therapies. Moving beyond this threshold for larger patient populations—toward 2,000 L scale or more—can be successfully achieved with smart, scalable, performance-driven materials, technologies, and supporting services at every step, from discovery stage to late stage clinical and commercial scales.

Process Development

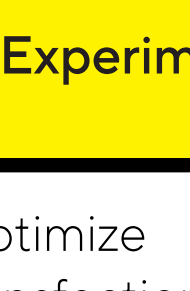
Media selection impacts cell growth, viability, and AAV productivity.

Adjust cell density, DNA load, and reagent ratio to maximize titers.

Fine-tune Helper/ RepCap/ Transgene ratios to improve packaging efficiency and % full capsids.



4Cell® HEK293 Media & Feed Portfolio



FectoVIR®-AAV



pPLUS® AAV Plasmids

Design of Experiments Using MODDE®

Optimize HEK293 cell culture

Optimize transfection step

Optimize transfection parameters & plasmid ratios

Upstream Optimization

Downstream Optimization

Optimize Harvest

Refine Purification Workflow

Strengthen Analytical Control

Accurately measure viral vector quality, including critical parameters such as percentage of full AAV capsids.

Optimize capture and polishing conditions: determine recovery, purity, and capacity.

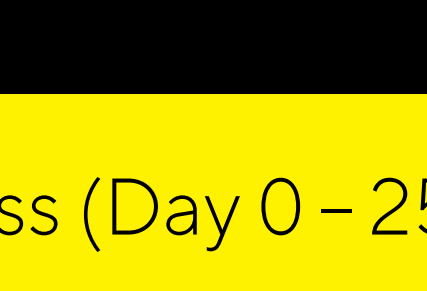
Consistent monitoring of full/empty ratio and viral genomes ensures product quality.



PATfix® AAV Switcher Platform



CIMmultus® Preparative Columns



PATfix® AAV Platform

Upstream Manufacturing Process (Day 0 – 25)

Seed Train Day 0 – 19

Early-stage amplification workflow



Thaw working cell bank vial

125 mL shake flask

1 L shake flask

N-1 500 L STR

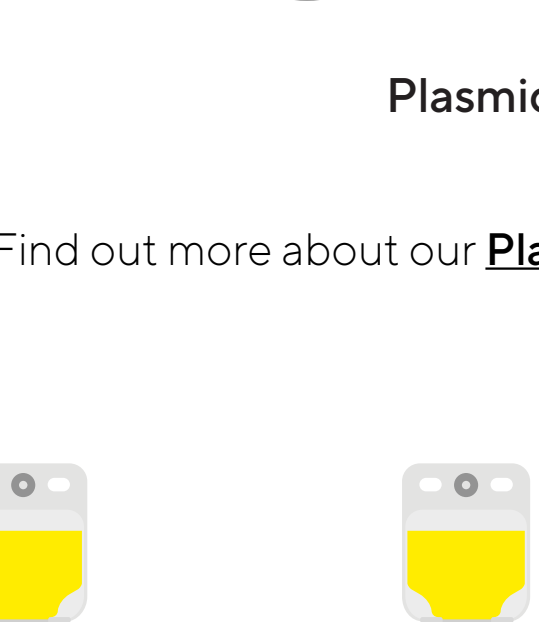
N-2 50 L rocker bag or STR

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

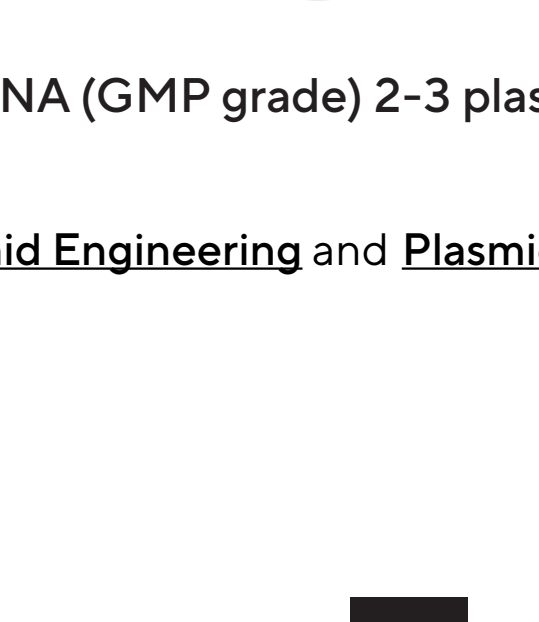
Transfection Day 19 – 25

Step 1:

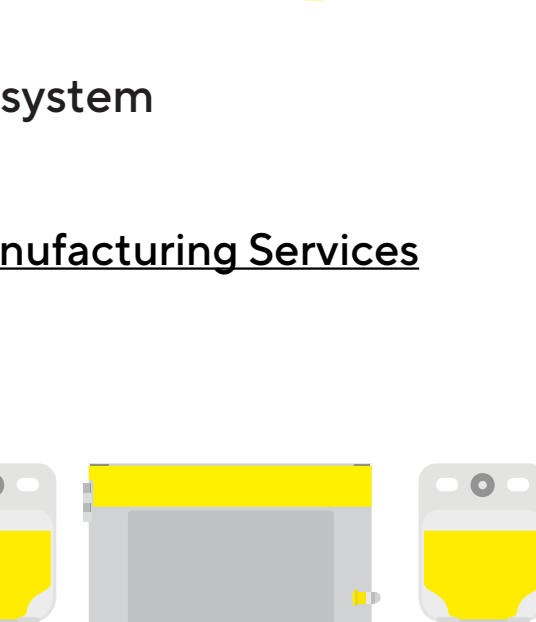
Prepare DNA solution by combining plasmid DNA with complexation media.



Transgene



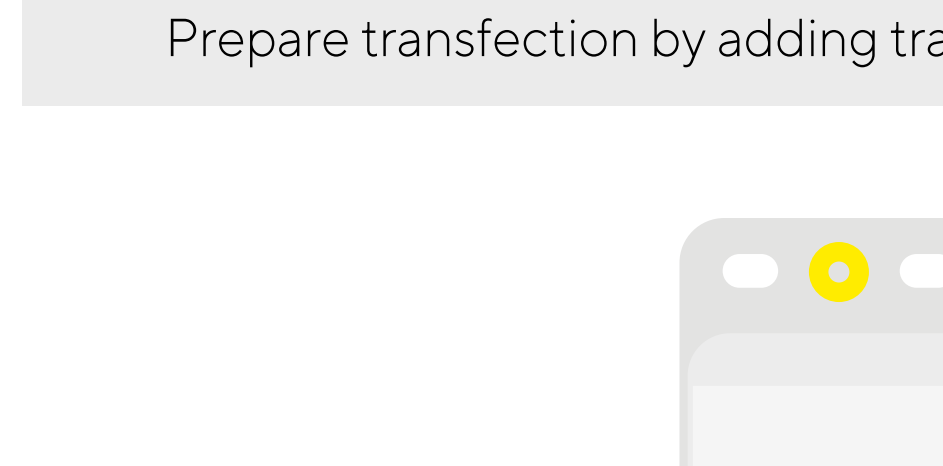
pPLUS® AAV-RC Range



pPLUS® AAV-Helper

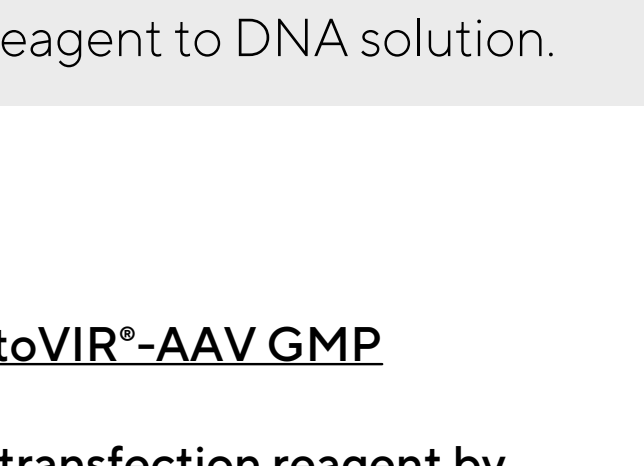
Plasmid DNA (GMP grade) 2-3 plasmid system

Find out more about our [Plasmid Engineering](#) and [Plasmid Manufacturing Services](#)



Biostat® RM and Flexsafe® bags

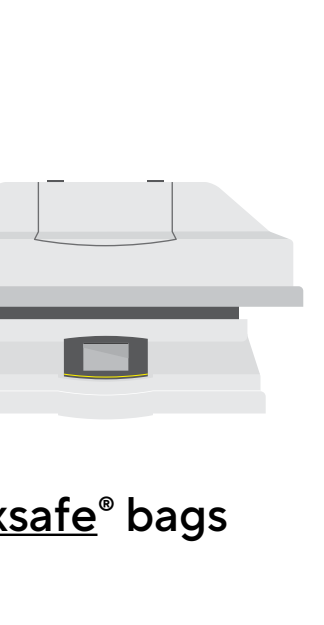
OR



Flexsafe® Pro Mixer

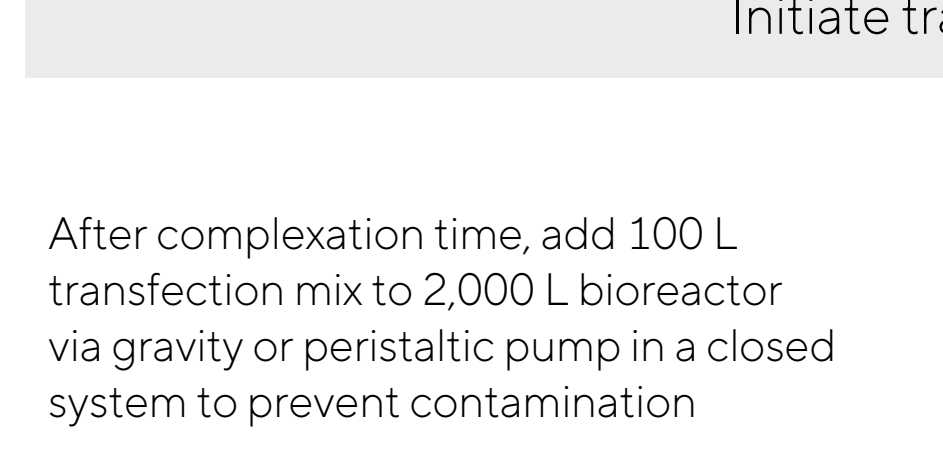
Step 2:

Prepare transfection by adding transfection reagent to DNA solution.



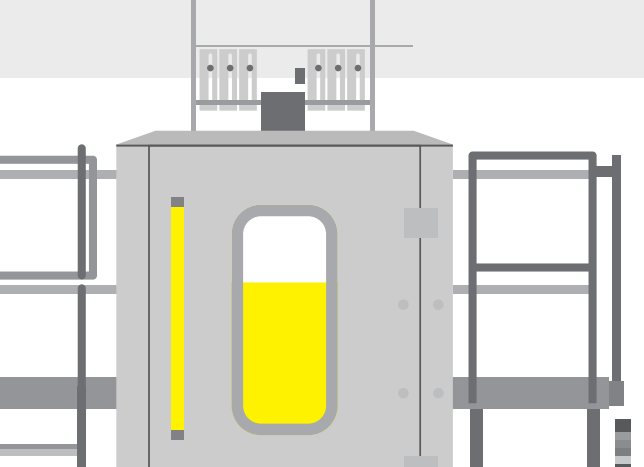
FectoVIR®-AAV GMP

Mix transfection reagent by inversion before use and add it pure directly to diluted DNA



Biostat® RM and Flexsafe® bags

OR



Flexsafe® Pro Mixer

Step 3:

Initiate transfection.

After complexation time, add 100 L transfection mix to 2,000 L bioreactor via gravity or peristaltic pump in a closed system to prevent contamination



Tips For Transfection Success:

- Maintain similar P/V between scales
- Include a shear protectant like Pluronic (up to 0.1%)
- Avoid overmixing complexes
- Avoid adding any base or antifoam within an hour of transfection
- Add 4Cell® HEK FS 2 feed post-transfection to further boost titers

Downstream Manufacturing Process (Day 25 – 28)

Harvest Day 24 – 26

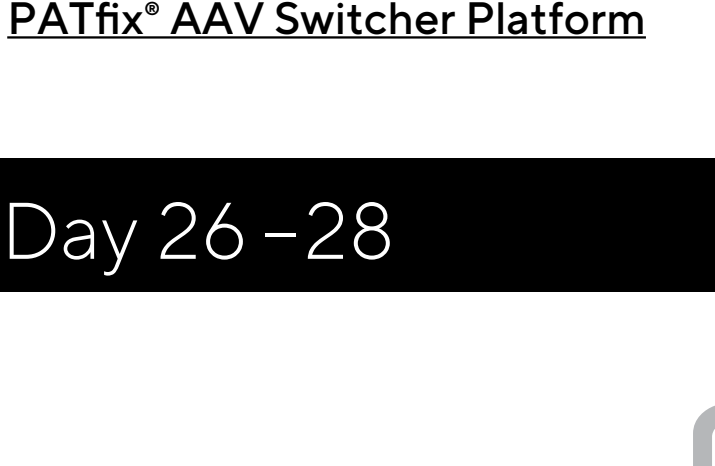
Collect crude harvest from bioreactor 48h or 72h post-transfection.

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

In-Process Analysis
Estimate empty/full capsid ratio with PATfix® AAV Switcher Platform, ELISA & qPCR/ddPCR/dPCR



Biostat® STR 2000L

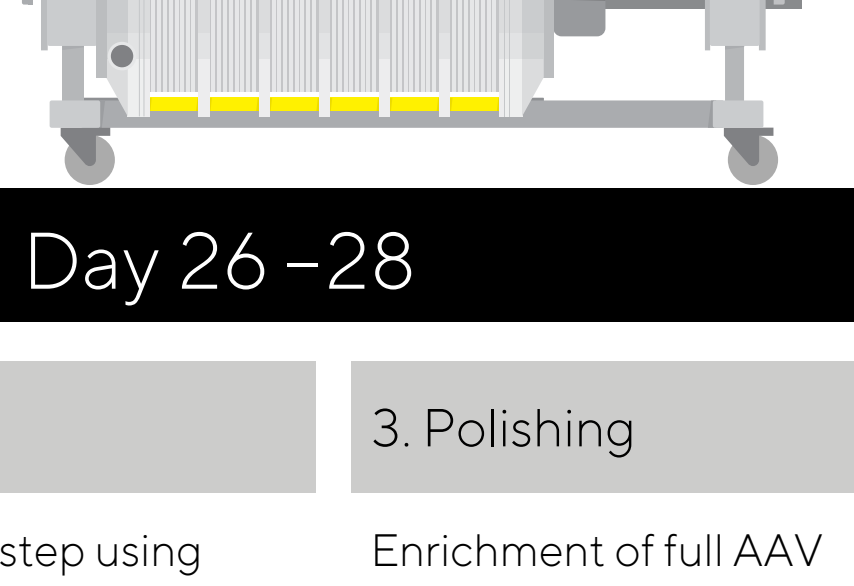


PATfix® AAV Switcher Platform

Clarification Day 26 – 28

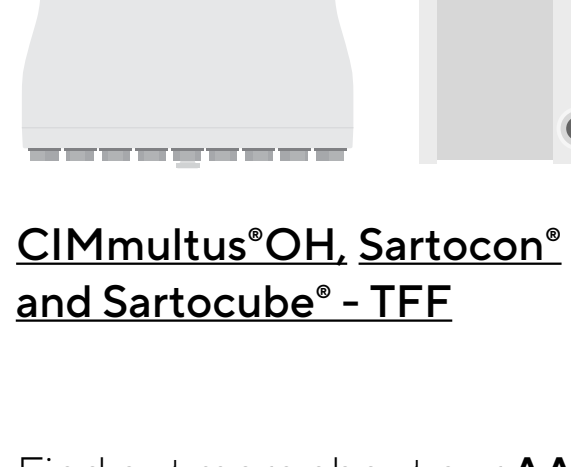
Microfiltration or depth filtration to remove contaminations generated during upstream process such as contaminating plasmid DNA, host-cell proteins, host-cell DNA, cell debris

Sartoclear® Depth filter

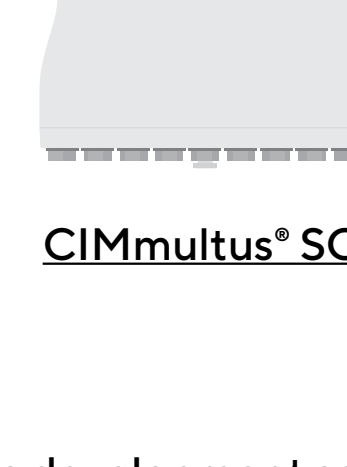


Purification Day 26 – 28

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|---|--|---|
| <p>1. Optional Precapture</p> <p>Concentrate the viral vectors using tangential flow filtration (TFF) or CIMmultus® OH</p> | <p>2. Capture</p> <p>AAV Capture step using serotype agnostic CIMmultus® SO3 column</p> | <p>3. Polishing</p> <p>Enrichment of full AAV capsids using anion exchange chromatograph: CIMmultus® QA HR or multimodal chromatography CIMmultus® PrimaT CIMmultus® PrimaS HR</p> |
|---|--|---|



CIMmultus® OH, Sartoclear® and Sartocube® - TFF



CIMmultus® SO3



CIMmultus® QA HR

Find out more about our [AAV process development services](#)

Final Formulation Day 26 – 28

Dose adjustment and TFF to final formulation, followed by sterile filtration using 0.2 µm filter

Sartoflow® 1,000



Fill & Finish Day 28



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

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

Find out more about our [Fill and Finish Solutions](#)

AAV Testing Services Day 28

Ensure product quality and regulatory readiness with end-to-end testing:

- | | | | |
|---|--|---|---|
| <p>Identity →
Capsid confirmation, gene of interest, peptide mapping</p> | <p>Purity →
Residuals, full: empty capsid ratio, aggregation & stability, capsid protein purity</p> | <p>Potency →
Genomic & infectious titers</p> | <p>Safety →
AAV replication competence, microbiological, viral</p> |
|---|--|---|---|

Find out more about our [AAV Testing Services](#)