Setting the Standard in rAAV Manufacturing: A Comparative Bioreactor Study Showcases the Strength of the EpyQ® STR Platform

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Introduction

Recombinant Adeno-Associated Virus (rAAV) vectors are shaping the future of gene therapy. Lowering production costs and boosting efficiency remain key to enabling broader access and driving market growth.

Using our proprietary split 2 plasmid system (EpyQ®), we consistently achieve >1×10^{11} vg/mL across multiple rAAV serotypes and scales (250 mL – 200 L) in stirred-tank bioreactors, demonstrating reliable, high-yield production. Although stirred tanks deliver strong performance in our hands, benchmarking against alternative bioreactor systems ensures we continue using the optimal platform. In our experience, orbital shaking and rocking bioreactors support robust HEK293 growth while applying lower power input to the cells, making them promising platforms for efficient rAAV manufacturing.

In this study, we compared rAAV9 production across three platforms - orbital shaker (SB10-X single-use Bioreactor; Kuhner), rocking bioreactor (ReadyToProcess™ WAVE 25 Rocker; Cytiva), and stirred-tank (Biostat® B 10 L single-use Bioreactor; Sartorius) - to evaluate how bioreactor design and operating conditions (shear, mixing, power input) affect cell growth, vector yield, and product quality.

rAAV Production & Quality Workflow

➢ Upstream

- Platforms: orbital shaking, rocking, and stirred-tank bioreactors
- Serotype: rAAV9
- Scale: 10 L (equal working volumes)
- Cell line: proprietary HEK293
- Transfection: EpyQ split 2 plasmid system

➢ Offline Monitoring

- Cell density & viability (ViCELL-BLU)
- Metabolic profiles (BioProfile® Flex2)

➢ Analytics

- Vector yields in crude lysates: vg titer (ddPCR), capsid yields (Gyrolab® immunoassay)
- % Full capsids (mass photometry, Refeyn)
- Encapsidated plasmid- and host-cell DNA-derived impurities (ddPCR)
- In-vitro vector potency relative to reference

Orbital Shaking

SB10-X single-use Bioreactor

Rocking Motion

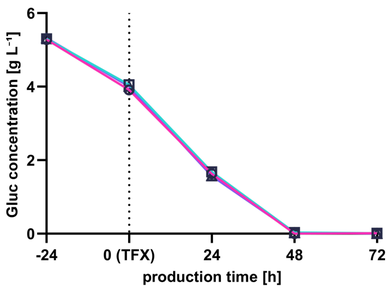
ReadyToProcess™ WAVE 25 Rocker

Stirring

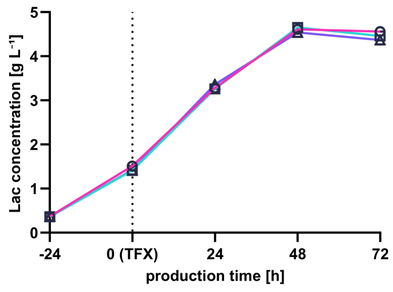
Biostat® B 10L single-use Bioreactor

Metabolite Trends

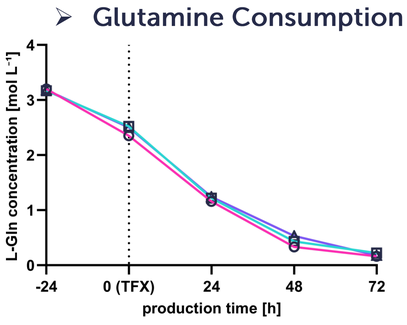
➢ Glucose Consumption



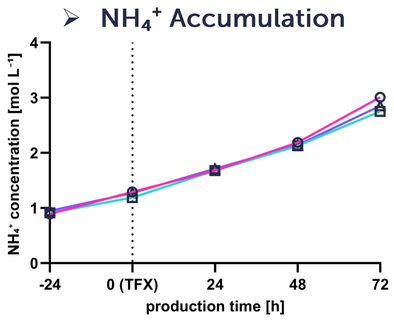
➢ Lactate Accumulation

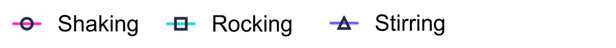


➢ Glutamine Consumption



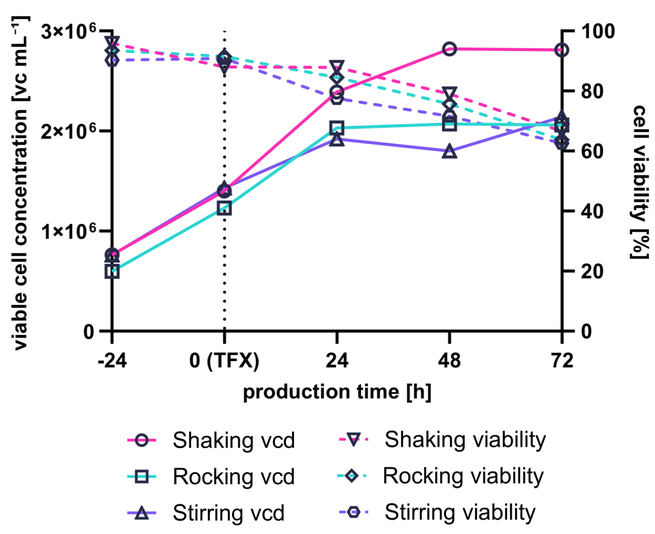
➢ NH_4^+ Accumulation





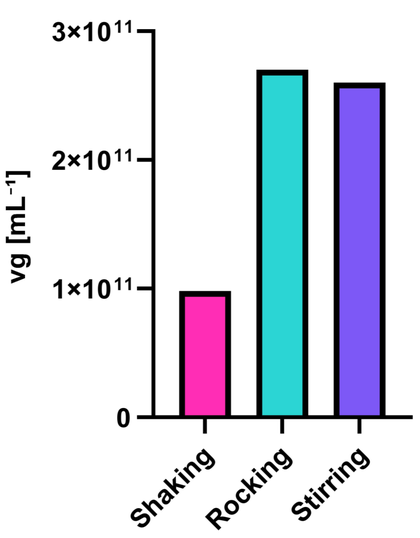
Growth and viability

➢ Viable Cell Density and Viability

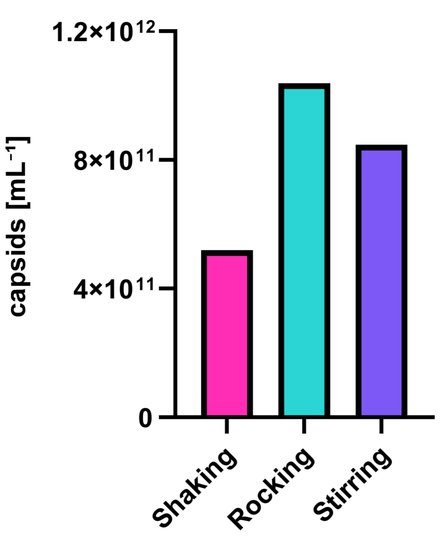


Crude Lysate Titers

➢ Vector Genome Yields

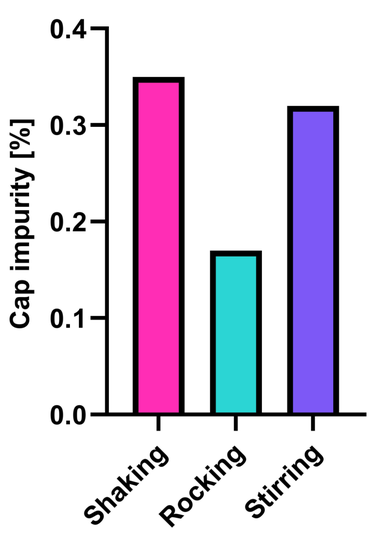


➢ Capsid Yields

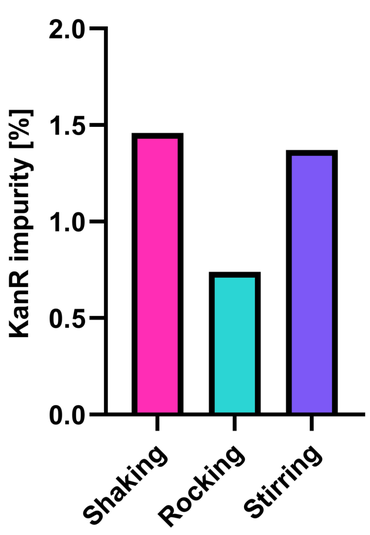


Encapsidated Impurity Profiles

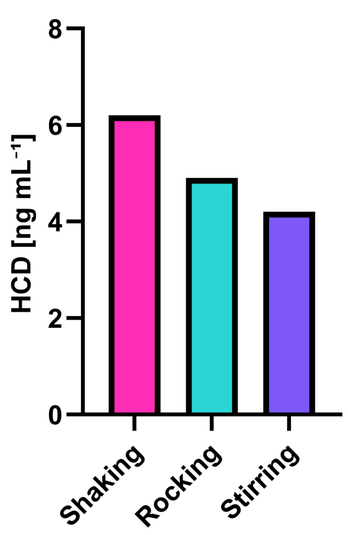
➢ Capsid Gene-Derived Impurities



➢ Kanamycin Resistance Gene-Derived Impurities

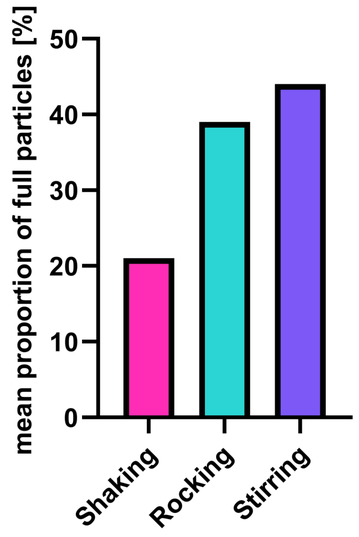


➢ Host-Cell DNA-Derived Impurities



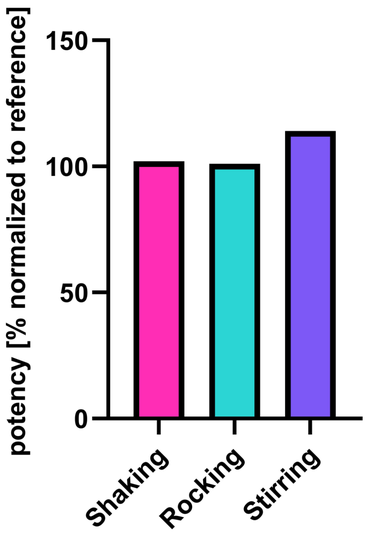
Full Capsid Yield

➢ Full/Empty Proportion



Biological Performance of the Drug Substance

➢ In Vitro Potency



Summary

Both stirred-tank and rocking bioreactors demonstrated excellent performance in rAAV production, clearly outperforming the shaken system in yield and product quality.

In-process data were largely consistent across platforms, with only the shaking system showing prolonged post-transfection growth, possibly reducing productivity.

While the rocking bioreactor showed strong results, its limited scalability confirms the stirred-tank bioreactor as our preferred platform for robust, large-scale manufacturing using our proprietary HEK293 cell line with the EpyQ platform.

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