SARTORIUS

Application Note

August 2025

Keywords or phrases:
AAV serotypes, Sartoclear Dynamics® Lab, Sartolab® Multistation, HEK293 cells, gene therapy, filtration optimization, diatomaceous earth, viral genome recovery, turbidity reduction, Ascend Advanced Therapies, CDMO, EpyQ® platform, process upscaling, bioreactor, filtration efficiency.

Optimizing AAV Serotype Filtration with Sartoclear Dynamics® Lab

Sirri Ndonwi[1], Beatriz L Carnio[1], Dila Bitnel[1], Jared Babic[1], Tatjana Fischer[1], Matthias Boscher[1], Johanna Wagner[1], Ahmed Youssef[1], Benjamin Graf[2], Andreas Pickl[2]

[1] Ascend Advanced Therapies GmbH, Planegg, Germany

[2] Sartorius Lab Instruments GmbH & Co.KG, Göttingen, Germany

* Correspondence

Email: ahmed.youssef@ascend-adv.com, LabFiltrationPM@sartorius.com

Abstract

In this application note we present data showcasing the clear filtration of different AAV serotypes using Sartoclear Dynamics® Lab in combination with the Sartolab® Multistation. Clarification experiments were first optimized in 250 mL batches and further upscaled to 1 L fractions. The production of the AAV material in HEK293 cells as well as the process optimization, analytical measurements and upscaling experiments were designed and performed by Ascend Advanced Therapies, a leading CDMO for high-quality, cost-effective gene and advanced therapy development and manufacturing. The further described results show that Sartoclear Dynamics® Lab can be used to simplify and accelerate the process of HEK lysate clarification and allows to reduce the hands-on time for clarification by a factor of ~6x compared to classical centrifugation followed by membrane-based clear filtration.

ascend

Introduction

Adeno-associated virus (AAV) vectors are increasingly utilized in gene therapy due to their ability to deliver genetic material with high precision and safety. However, the production of AAV vectors is a complex and time-consuming process with various steps in the downstream processing. To ensure the release of all AAV particles produced in a cell culture suspension, the cells are usually lysed by a chemical or physical lysis step. This lysis leads to contamination of the AAV containing lysate material with cell debris, DNA and other impurities. Therefore, the first step of the AAV purification workflow is a clarification of the AAV lysate. Usually this is achieved by a combination of a prior centrifugation to remove most of the cell debris, followed by a classical dead-end filtration using a vacuum driven bottle top filter with a filtration membrane. However, this process is time-consuming due to the duration of the centrifugation process and often not robust due to filter clogging.

To simplify and streamline this first clarification step, Sartoclear Dynamics® Lab (SDL) was developed. SDL contains diatomaceous earth (DE), as a filter aid material which is added to the AAV lysate solution prior to application onto a filter membrane. The DE protects the filter membrane from blocking which allows to circumvent the time-consuming centrifugation step. Moreover, we developed the Sartolab® Multistation, an adapter that allows the parallel filtration with up to 6x Sartolab RF|BT® bottle top filters.

In this application note we are showing real-life data of SDL filtrations. The experiments and results presented in the application note were performed by Ascend Advanced Therapies, a leading CDMO for high-quality, cost-effective gene and advanced therapy development and manufacturing, from clinic to commercialization.

As a first step, different amounts of DE were added to AAV9 lysate material to titrate the optimal amount of DE for the filtration process. In further steps, the process was upscaled for the filtration of larger volumes. In total three different serotypes (AAV9, AAV5 and AAV2) were successfully produced in high-titer solutions and clarified using SDL without significant loss in viral genome titer and high reduction of the turbidity.

AAV Production with Ascend's EpyQ® Platform

Our study focused on the production of AAV using HEK293 cell cultures in conjunction with Ascend's proprietary EpyQ® platform. This platform incorporates efficient split-2 plasmids specifically designed to optimize both the yield and quality of AAV.

All AAV serotypes were generated in a controlled bioreactor environment, with scales ranging from 250 mL to 10 L bench-scale bioreactors. Following production, the material underwent either chemical lysis or Ascend's proprietary mechanical lysis method, each followed by endonuclease treatment before final filtration.

Results

Titration of DE amount

To evaluate the amount of DE required for a fast filtration process without significant loss of AAV particles, different amounts of DE were added to 250 mL AAV9 lysate solutions. As Figure 1 A shows, an increasing amount of DE added prior to filtration leads to a reduction of the filtration time. For all conducted filtrations, irrespective of the DE amount added the filtration was successful and did not lead to a complete blockage of the filter membrane. However, the filtration time was significantly reduced by a factor of 23.6x from 1770 s (4 g/L DE) down to 75 s (20 g/L DE). Analytical measurements using digital PCR revealed a high viral genome (VG) recovery for all filtrations with different amounts of DE added. Figure 1 B shows that for all conducted filtrations a high turbidity reduction was reached (log reduction values between 1.6x and 1.4x). For the fastest filtration using 20 g/L DE, the turbidity was reduced from 393.7 NTU to 17.3 NTU which corresponds to a reduction factor of ~22.7x.

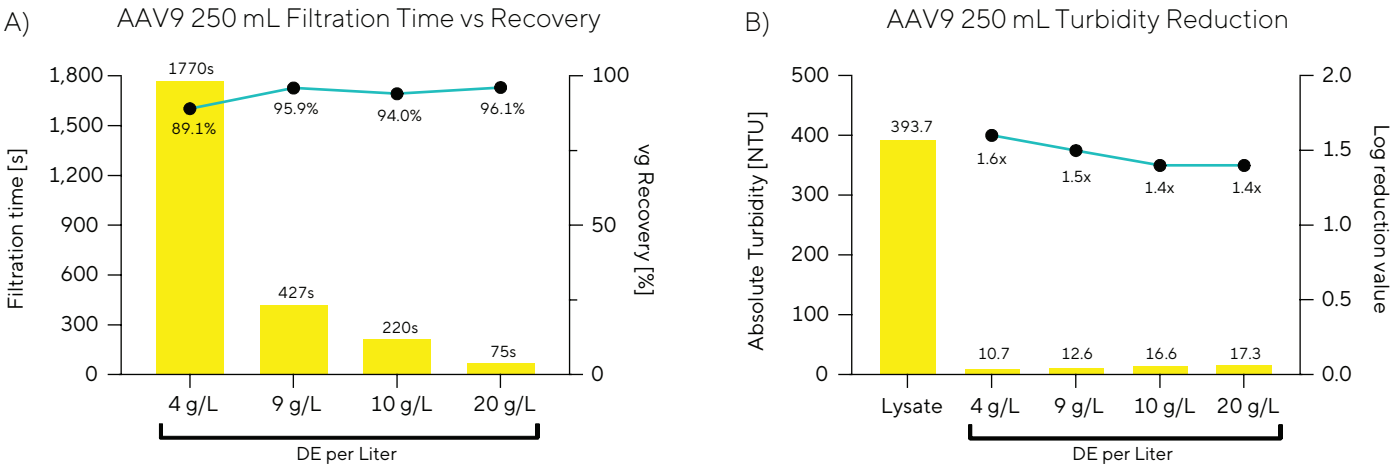

Figure 1: *Titration experiment for SDL clarification of HEK293 AAV9 lysate. A) Titration experiment with increasing amounts of pre-wetted DE added to 250 mL HEK293 AAV9 lysate solutions showed that an increase in the SDL concentration results in a significant reduction of the filtration times (yellow bars). Analytical measurements using ddPCR showed that an increase in the amount of DE did not result in a reduction of the viral genome recovery (black dots). B) Turbidity measurements of the 250 mL filtrations with increasing DE amount showed only a minor increase in the turbidity (yellow bars). The log reduction value for all tested filtration was found to be in a range between 1.6x (4 g/L) and 1.4 x (10 g/L and 20 g/L).*

Upscaling to 1 L Filtrations

After successful filtrations of 250 mL AAV 9 cell lysate material, the clarification process was upscaled to a volume of 1 L. Figure 2 A shows the filtration of 2 × 1 L solutions with two different amounts of DE added (11.25 g/L and 22.5 g/L). The use of 22.5 g/L DE resulted in a total filtration time of around 4 min (237 s). Analytical results showed that high viral genome recoveries between 93.0% and 98.9% for both performed filtrations, respectively. This indicates that only a minor amount of filled AAV9 particles are lost during the filtration process using SDL. Additional turbidity measurements showed that the filtration process results in a turbidity reduction from 414 NTU down to 13.6 and 13.4, respectively (see Figure 2 B). This corresponds to a log reduction value of ~1.5 for both amounts of DE tested.

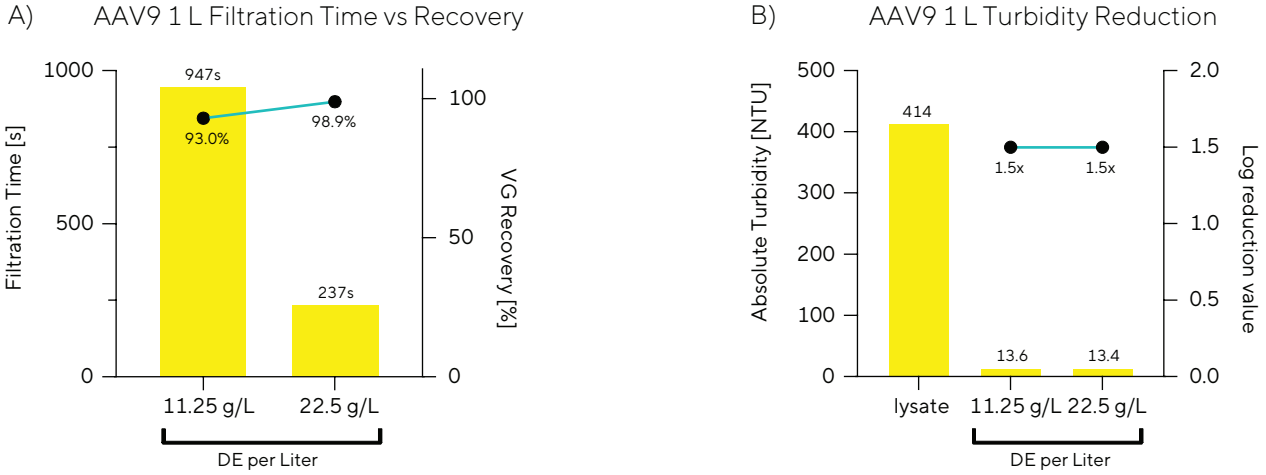

Figure 2: *Filtration of 1 L HEK293 AAV9 lysate using Sartoclear Dynamics® Lab. A) The titration of two different amounts added to 1 L AAV9 lysate shows a significant improvement of the filtration time if 22.5 g/L instead of 11.25 g/L DE are added to a AAV9 lysate solution (yellow bars). The increase in the absolute DE amount added did not have a negative impact on the recovery of viral genomes (black dots). B) Turbidity measurements before and after the filtration using 11.25 g/L or 22.5 g/L DE showed that the total amount of DE added does not have any impact on the turbidity reduction. For both filtrations the turbidity was reduced from 414 NTU (left yellow bar) to 13.6 NTU (middle yellow bar) and 13.4 NTU (right yellow bar), respectively. For both clear filtrations tested, the log reduction value had a factor of 1.5x (black dots).*

Filtration of Different Serotypes

After successful filtration of AAV9 lysate material, the two other serotypes AAV5 and AAV2 were used for 1 L filtrations using DE as a filter-aid material. For the filtrations a total amount of 22.5 g/L DE was added. Figure 3 A shows that all filtrations were finished under 5 min with a maximum time of 4.45 min (267 s) for AAV5. Analytical measurements showed that none of the tested serotypes, filled AAV particles, were lost during the filtration process. The viral genome recoveries were found to be 98.9%, 94.8% and 98.7%, respectively.

Additional measurements of the turbidity reduction showed similar results as those of AAV9 (see sections above). Figure 3 B shows that for all three tested AAV serotypes the turbidity was significantly reduced by a log reduction value between 1.5 and 1.7. The AAV9 and AAV5 lysate solutions had a NTU of above 400 and were reduced to 13.4 NTU after the DE-filtration. The AAV2 lysate had a lower turbidity of 243 NTU and was reduced to 7.3 NTU via DE-filtration.

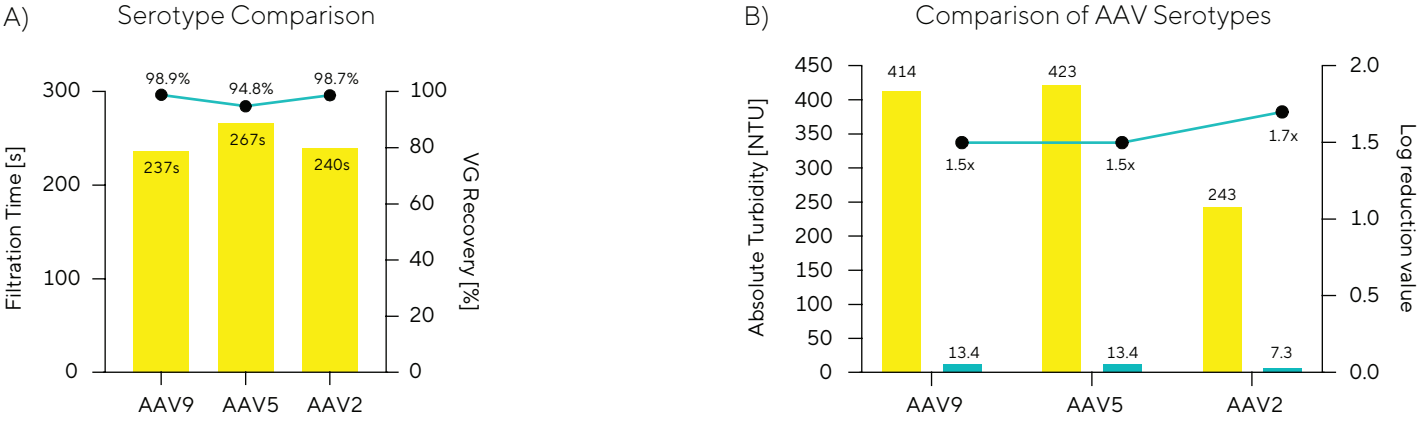

Figure 3: *Back-to-back comparison of 1 L clarifications using Sartoclear Dynamics® Lab with three different AAV serotypes. A) Filtration processes of the three tested serotypes AAV9, AAV5 and AAV2 all took less than 5 min (300 s, yellow bars). Analytical measurements using ddPCR showed that a maximum of 5.2% of the viral genomes were lost (AAV5) during the filtration process. For AAV9 and AAV2 only 1.1% and 1.3% of the viral genomes were lost during filtration, respectively. B) Turbidity measurements showed that the turbidity of all three tested AAV serotypes was significantly reduced by a log reduction value of 1.5x (AAV9 and AAV5) and 1.7x (AAV), respectively (black dots). In absolute numbers, the AAV9 lysate turbidity was reduced from 414 NTU (yellow bar) to 13.4 NTU (teal bar), AAV5 from 423 NTU to 13.4 and AAV2 from 243 NTU to 7.3 NTU.*

Filtration Time

In lab scale processing of volumes up to ~10 L, a common and widespread protocol for clarification of AAV lysate material is a combination of a first centrifugation step, followed by a dead-end filtration using vacuum-driven bottle top filters. The centrifugation step is required to remove cell debris as well as aggregates of insoluble protein and other unspecific process related impurities. Without this step, the filter membrane is constantly blocked, preventing a filtration of the whole AAV lysate solution. To solve this problem, SDL was developed. The diatomaceous earth which is pre-mixed with the AAV lysate solution prior to application onto a filter membrane, allows complete filtration without filter blocking. This in turn means that the whole clarification process can be shortened by the whole centrifugation protocol and can be covered by a simple vacuum-driven clear filtration step.

Figure 4 shows a comparison of the complete processing time required for the clarification of 4 × 250 mL (1 L in total). While the combination of centrifugation + subsequent filtration takes 30 min, the process can be shortened by factor of 6x to 5 min using SDL. In combination with the Sartolab® Multistation a parallelized filtration of up to 6 × 1 L AAV lysate material can be achieved – even under a laminar flow hood (Figure 5). It should also be noted that even higher AAV lysate volumes can be covered by subsequent use of the Sartolab® Multistation in combination with fresh units of SDL units.

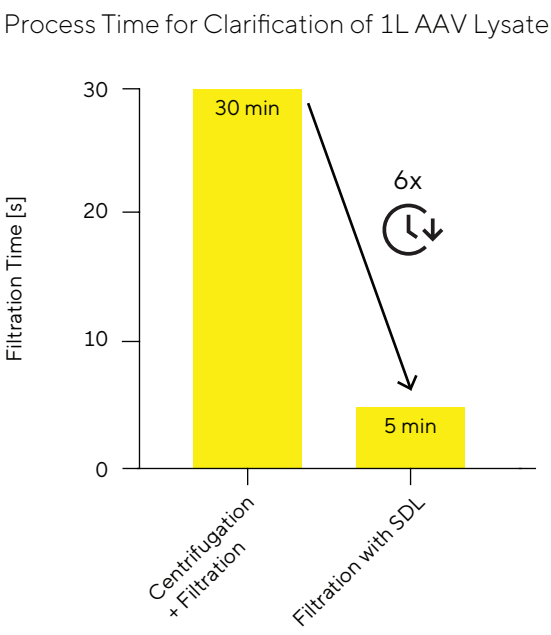

Figure 4: *Comparison of processing time of 1 L HEK293 AAV9 lysate material. The classical clarification process that is based on the combination of centrifugation followed by clear filtration using standard bottle top filters without additional filter aid material results in a processing time of 30 min. By using Sartoclear Dynamics® Lab with 1,000 mL Sartolab® RF bottle top filters, the clarification process is shortened by a factor of 6x to 5 min.*

Figure 5: *Sartolab® RF 1000 + Multistation: In combination with the Sartolab® Multistation up to 6 × 1 L can be processed in parallel. The Sartolab® RF|BT filter units are available in different formats: 50 mL, 150 mL, 250 mL, 500 mL and 1,000 mL.*

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
3874 Research Park Drive
Ann Arbor, MI 48108
Phone +1 734 769 1600

For further information, visit
sartorius.com

Status: 08 | 2025