# Successful capture DoE studies for a range of AAV serotypes to reduce manufacturing costs & accelerate development

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## Abstract

AAV is nowadays the most widely used virus for in vivo gene therapy. To achieve high vector potency in specific target cells or organs, to reduce off target transduction and to overcome immunological barriers in AAV gene therapy, many naturally occurring AAV serotypes and isolates can be chosen. Further, in the past about 2 decades, many capsid engineering technologies have been developed to further tune AAV capsids for a desired application. Capsid choice can impact both, upstream and downstream manufacturing of AAV vectors and necessitates capsid specific assay development. Starting material design and transfection optimization are the main parameters to be considered in upstream. For downstream processing, capsid specific adaptions are crucial in each process step starting from capture where affinity chromatography is commonly used.

Here we show for serotypes AAV2, AAV5 and AAV8 how product recovery and vector quality are affected by varying the elution buffer conditions of the affinity step. For each serotype, the best elution pH and conductivity were determined using a design of experiment (DoE) approach. The capsid load was found to be non-significant within the tested range. In addition, the possible effects of the optimal affinity elution buffer conditions were evaluated with respect to a subsequent full empty polishing step and compatibility was confirmed.
It was found that AAV5 behaved very differently compared to the other serotypes in terms of elution conditions. Finally, selecting an affinity resin that allows a wide dynamic range for capsid loading with a high affinity has shown to be key to reducing manufacturing costs and developing a robust capture step to provide safe vectors at reduced manufacturing costs.


## Optimization DoE Design

**Factors**

- Elution conductivity
- Elution pH
- Capsid load (only AAV5)

DoE

**Responses**

- Capsid recovery (yield)
- Capsid intensity (% monomer)

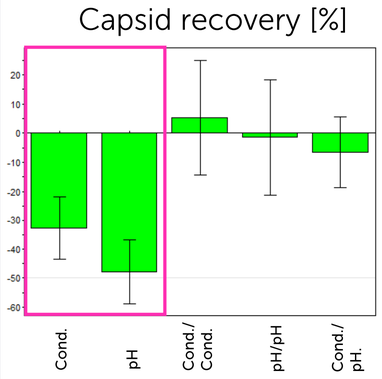
Three DoE studies were performed to achieve best affinity chromatography elution conditions for the serotypes AAV2, AAV5 and AAV8. The DoE factors conductivity and pH of the elution buffer were varied during the study. Capsid titer was measured for capsid recovery determination and % monomer as indicator for undesired aggregation via capsid intensity. Both read-outs were determined by Stunner® (Unchained Labs) in this study.

Using this workflow, high throughput analysis is possible which enables the performance of DoE studies in a very short time. We can identify best conditions for each specific serotype to increase recovered capsids and to reduce potential capsid aggregation during affinity chromatography which could negatively impact further downstream processing and overall yield and quality.

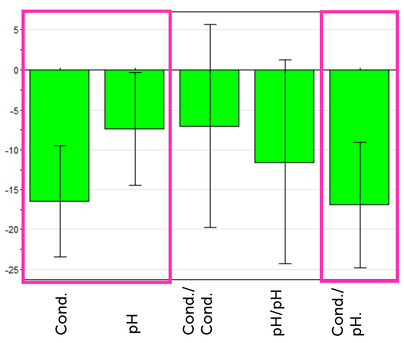
## Results AAV5

### Coefficient plots

Capsid recovery [%]



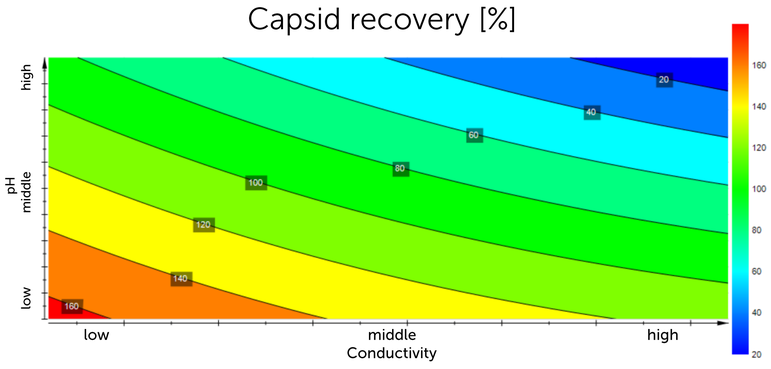

Capsid intensity [%]



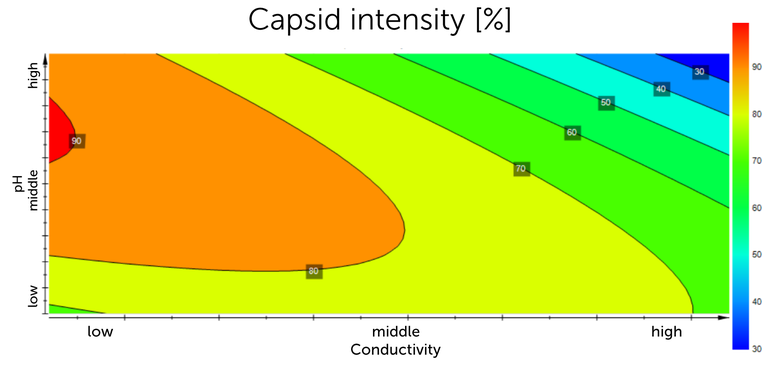

➢ Elution conductivity and pH (see pink frame) have a significant influence on capsid recovery and intensity. Capsid load was not significant. Therefore, it was removed.

### Contour plots

Capsid recovery [%]




Capsid intensity [%]

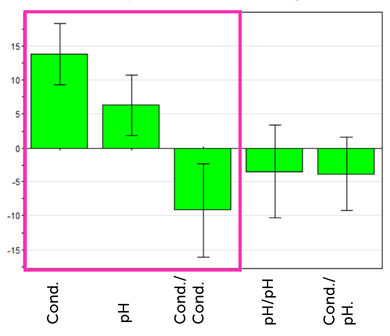



➢ Low pH and conductivity is beneficial for a high capsid recovery, but for a low aggregation level (high capsid intensity) a middle to high pH is necessary.

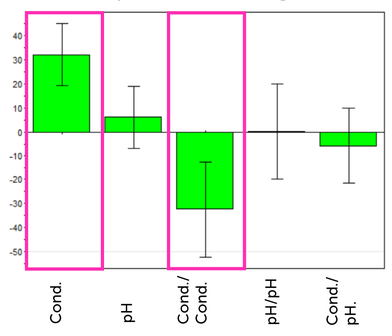
## Results AAV2

### Coefficient plots

Capsid recovery [%]



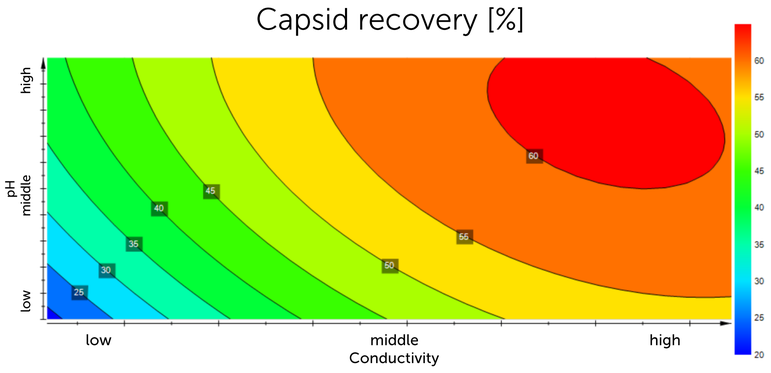

Capsid intensity [%]



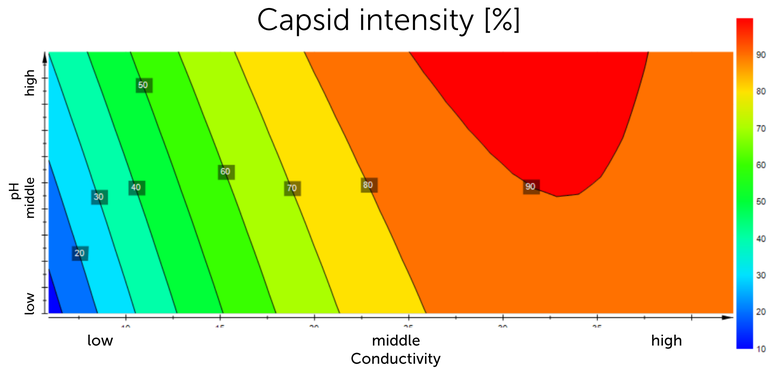

➢ Elution conductivity and pH (see pink frame) have a significant influence on capsid recovery. For capsid intensity only conductivity is significant.

### Contour plots

Capsid recovery [%]




Capsid intensity [%]

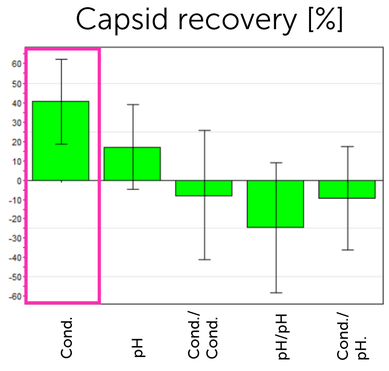



➢ High pH and conductivity is beneficial for a higher capsid recovery and more capsid monomers.

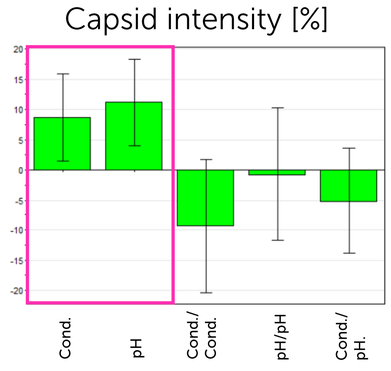
## Results AAV8

### Coefficient plots

Capsid recovery [%]



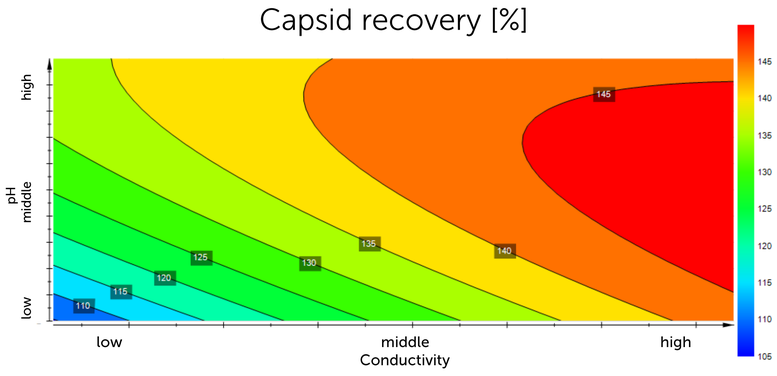

Capsid intensity [%]



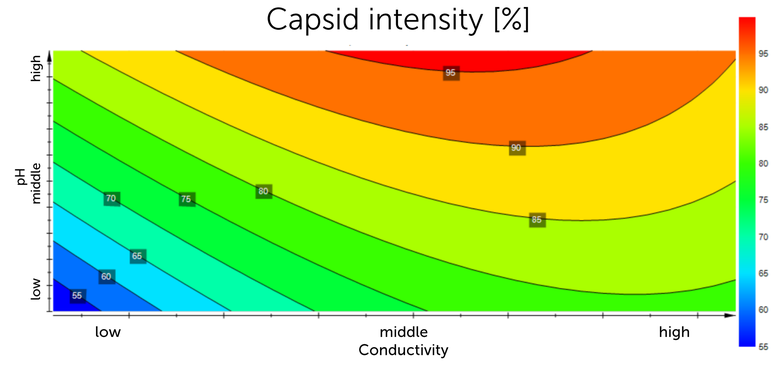

➢ Elution conductivity (see pink frame) has a significant influence on capsid recovery. For capsid intensity elution conductivity and pH are significant factors.

### Contour plots

Capsid recovery [%]




Capsid intensity [%]




➢ High pH and conductivity is beneficial for a higher capsid recovery and capsid monomers.

| Serotype | Affinity chromatography conditions |
|---|---|
| AAV5 | Low pH and low conductivity |
| AAV2 | High pH and high conductivity |
| AAV8 | High pH and high conductivity |

## Summary

Different AAV serotypes require different elution conditions in order to improve capsid recovery and to reduce aggregation. It was determined that AAV5 behaved differently compared to AAV2 and AAV8. A low elution pH and a low conductivity is required for AAV5 whereas a high pH and conductivity is necessary to achieve the best results for AAV2 and AAV8.
With the chosen analytics and workflow, we can accelerate DoE based DSP optimization for our clients.
Further studies showed that selecting an affinity resin with a wide dynamic binding range and a high affinity is key to reduce manufacturing costs and developing a robust capture step to provide safe vectors at reduced manufacturing costs.

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