Accelerating advancement in gene therapy by improving downstream purification of viral vectors

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INTRODUCTION
Adeno-Associated Virus (AAV) has become the vector of choice in many gene therapies. Recent advances in chromatography resin development for viral vectors have demonstrated that AAV purification can be scalable and efficient, achieving high purity and yield in a single step. With an extensively growing pipeline of gene therapy clinical trials it is evident that scalable production solutions are needed. Here we outline the benefits of implementing affinity chromatography in the downstream purification of viral vectors.

AFFINITY SOLUTIONS FOR VIRAL VECTOR PURIFICATION

<table>
<thead>
<tr>
<th>Thermo Scientific™ resin</th>
<th>Binding Capacity (vg/mL)</th>
<th>Serotype Affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>POROS™ CaptureSelect™ AAV8</td>
<td>&gt;10¹¹</td>
<td>AAV8</td>
</tr>
<tr>
<td>POROS™ CaptureSelect™ AAV9</td>
<td>&gt;10¹¹</td>
<td>AAV9</td>
</tr>
<tr>
<td>POROS™ CaptureSelect™ AAVX</td>
<td>&gt;10¹¹</td>
<td>AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, recombinant &amp; chimeric vectors</td>
</tr>
</tbody>
</table>

- Affinity through antibody selectivity (CaptureSelect™ technology) : technology based on Camelfield-derived single domain [VHH] antibody fragments.
- Animal origin-free production process (Sacharomyces cerevisiae)
- Combined with the large through-pore POROS backbone

Fig.1 CaptureSelect™ ligands are VHH fragments (single domain antibody fragments – sdAb), the smallest antigen binding molecule.

The small size of VHH fragments (15kD) allows binding at difficult to reach epitopes. Overall, VHH fragments offer high specificity, affinity and stability.

Combining antibody-based selectivity and process robustness in unique AAV affinity resins

ENABLING INDUSTRIAL SCALE DEVELOPMENT OF AAV VECTORS

CaptureSelect™ Paradigm

- Affinity capture = fewer chromatography steps
- Simpler process
- Lower cost & speed to market
- Fewer steps = higher yield

Customer testimonial: “Process yield improvement from 20% to 60% & cost reduction by a factor of 10”

Fig. 2 Reduced number of process steps through AAV affinity chromatography resins

Reduce the number of steps in a purification procedure without compromising product purity and yield

Increase process flexibility and throughput

UNDERSTANDING THE VIRAL CLEARANCE POTENTIAL

Viral clearance data from an AAV8 clinical production process using the AAVX resin.

<table>
<thead>
<tr>
<th>RNA</th>
<th>DNA</th>
<th>RNA</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values</td>
<td>4.6</td>
<td>3.6</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Table 1. Clearance of model viruses. Log reduction values (LRV) of enveloped (env) and non-enveloped (non-env) viral models using the AAVX resin.

The AAVX resin can be an effective viral clearance step in the downstream process of AAV production

POROS™ CAPTURESELECT™ AAVX RESIN: A TRUE PLATFORM FOR AAV PURIFICATION

- Broad selectivity to both natural and synthetic capsids
- High dynamic binding capacity
- High elution recovery at different flow rates
- Robust, with less process optimization

AAV SEROTYPE SPECIFICITY

To date, the AAVX ligand has shown affinity towards all serotypes tested

BREAKTHROUGH ANALYSIS AND ELUTION RECOVERY

Fig. 3 Vector purity. A 1-step Compatible purity, as determined by SDS page, was obtained between a 1-step AAV9 affinity step on 3 IEX steps.

Fig. 4 Vector recovery. Yields > 80% were obtained from 10 L tot 200 L scale.

Fig. 6 AAVX resin serotype specificity with a large variety of serotypes.

Fig. 7 AAVX Breakthrough analysis. Breakthrough as a function of vg loaded per ml of resin. The breakthrough stayed below 0.5% up to 2e13 VG/ml of resin.

Fig. 8 Elution recovery. Elution recovery was above 80% at each of the three different flow rates used. Recovery was not influenced by flow rate or residence time.

- High capacity and a high degree of process design flexibility
- High elution recovery at different flow rates

DEMONSTRATING SCALABILITY

Use of AAVX at various scales shows:

- Robust resin scale up
- Comparable recoveries at various scales
- Consistency in resin performance

CONCLUSIONS

POROS™ CaptureSelect™ AAV resins address the current challenges involved with viral vector purification. Use of these resins will:

- Simplify your purification process and increase process design flexibility
- Allow for scalable purification of multiple AAV serotypes in a single platform

In addition we have demonstrated that the AAVX resin can be an effective viral clearance step in the downstream process of AAV manufacturing

TRADEMARKS/LICENSING

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