CIM Convective Interaction Media®
APPLICATION NOTE

A027 Single-step Method for Purification of Bacteriophage VDX-10 on CIM® QA Disk Monolithic Column and Method Scale-up

Bacteriophages are used in a broad range of applications, including phage therapy and phage display. With the growing problem of antibiotic resistance leading to untreatable bacterial infections, they are becoming very interesting as antimicrobial agents, not only in medicine, but also in veterinary medicine, food industry and agriculture. Phages intended for use as antimicrobial agents, especially those for human use, need to be purified of contaminants.

Here we present efficient single step purification method for a Staphylococcus aureus phage VDX-10 from bacterial lysate on a CIM® QA Disk Monolithic Column (Figure 1). The described method can be used also on a larger scale using a CIM® QA-8 mL Tube Monolithic Column (Figure 2).

**Figure 1. Purification of VDX-10 phage on a CIM® QA Disk Monolithic Column.**

VDX-10 phages were produced in the host strain Staphylococcus aureus. The Bacteria culture was incubated with shaking at 37 °C (99 °F) to mid-log phase, infected with phages at low multiplicity of infection and incubated with shaking until lysis was observed. The lysate was centrifuged and the supernatant was passed through a 0.45 micron filter. This cleared and filtered bacterial lysate was diluted with Buffer A and loaded on a CIM® Monolithic Column.

**Conditions for Figure 1 and 2:**
- **Column:** CIM® QA Disk Monolithic Column (3 x 12 mm I.D., CV: 0.34 mL)
- CIM® QA-8 mL Tube Monolithic Column (Do: 15 mm, Di: 1.5 mm, L: 45 mm, CV: 8 mL)
- **Sample:** Staphylococcus aureus lysate containing phage VDX-10
- **Loading volume:** 11 mL (for disk) and 470 mL (for 8 mL tube) of bacterial lysate containing VDX-10 phages, diluted with the binding mobile phase 1:1 (v/v) in Buffer A
- **Mobile phase:**
  - Buffer A: 100 mM phosphate buffer, pH 7.0
  - Buffer B: 100 mM phosphate buffer containing 2 M NaCl, pH 7.0
- **Conditions:** Step gradient at 0.6 M NaCl and 2 M NaCl in Buffer A
  - Flow rate: 212 cm/h (4 mL/min for disk and 30 mL/min for 8 mL tube)
  - UV detection at 280 nm
- **Titer determination:** Infective virus particles were determined by plaque assay.
- **Protein quantification:** Bradford Ultra Assay (Expedeon).
- **Host cell DNA quantification:** TaqMan® Staphylococcus aureus Detection Kit (Applied Biosystems).
Single step purification method for *Staphylococcus aureus* VDX-10 phage on a CIM® QA Disk Monolithic Column results in more than 99% of host cell DNA and more than 90% of proteins removal with 60% recovery of viable phages. Comparable results were obtained when the purification method was scaled-up from a CIM® Monolithic Disk to a larger CIM® QA-8 mL Tube Monolithic Column (Table 1). The dynamic binding capacity for VDX-10 phages is 1.1x10^10 pfu/mL of CIM® monolith.

**RESULTS**

The single step purification method for *Staphylococcus aureus* VDX-10 phage on a CIM® QA Disk Monolithic Column results in more than 99% of host cell DNA and more than 90% of proteins removal with 60% recovery of viable phages. Comparable results were obtained when the purification method was scaled-up from a CIM® Monolithic Disk to a larger CIM® QA-8 mL Tube Monolithic Column (Table 1). The dynamic binding capacity for VDX-10 phages is 1.1x10^10 pfu/mL of CIM® monolith.

More details can be found in the following article:

**Table 1. Virus recovery and impurities removal on CIM® QA Disk Monolithic Column and CIM® QA-8 mL Tube Monolithic Column.**

<table>
<thead>
<tr>
<th></th>
<th>Virus recovery</th>
<th>Protein removal</th>
<th>Host cell DNA removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIM® QA Disk Monolithic Column</td>
<td>54 %</td>
<td>90 %</td>
<td>&gt;99 %</td>
</tr>
<tr>
<td>CIM® QA-8 mL Tube Monolithic Column</td>
<td>65 %</td>
<td>91 %</td>
<td>&gt;99 %</td>
</tr>
</tbody>
</table>

**Figure 2: Purification of VDX-10 phage on a CIM® QA-8 mL Tube Monolithic column.**

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CIM® technology is covered by US patents 4889632, 4923610, 4952349 and 5972218. Other patents pending.

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