Recombinant baculovirus is extensively used for expression of recombinant proteins in insect cells. The appeal of baculovirus systems lies in their high level expression within an eukaryotic system, providing target proteins with appropriate posttranslational modifications. Recent approaches as vector in human gene therapy applications indicate a new dedication for baculovirus.

In any field of operation the increasing demand of highly pure baculovirus requests efficient, robust and scaleable purification strategies. Traditional techniques such as ultracentrifugation and tangential flow filtration are efficient in terms of virus concentration, but suffer from low yield and clearly lack robustness and scalability. In this application sheet we introduce a CIM monolith based purification process for infective baculovirus.

The protocol provides high recovery of active virus, efficient removal of host cell impurities, ease of use and straightforward scale up.

SAMPLE AND METHODS:

Column: CIM® QA-1 Tube Monolithic Column (Quaternary amine; CV: 1 mL)
CIM® Epoxy-1 Tube Monolithic Column (Epoxy; CV: 1 mL)
Mobile phases: Buffer A: 50 mM HEPES, 200 mM NaCl, pH 7.2
Buffer B: 50 mM HEPES, 1 M NaCl, pH 7.2
Regeneration buffer: 2 M NaCl, 1 M NaOH
Flow rate: 1 mL/min
Method: Step gradient elution: 0-10-30-50-100% B
Step hold volume: 15 CV
Sample: Baculovirus AcMNPV, produced in Sf9 cells
Sample volume: 300 mL
UV detection: 260 and 280 nm

> Figure 1
Purification scheme of baculovirus containing Sf9 supernatant, featuring direct load of filtered supernatant on CIM® monolith

Analysis of process samples was performed by SDS-PAGE and Western Blot for detection of virus, plaque forming assay for quantification of infective virus, Quant-iT PicoGreen Assay (Invitrogen) for determination of total DNA and Coomassie blue G-250 based protein assay (BioRad Laboratories) for determination of total protein.
RESULTS:

Purification of infective baculovirus on CIM® monolith was successfully performed by two complementary chromatography protocols:
1) Process version 1 - comprising a single column purification on CIM® QA.
2) Process version 2 - employing CIM® Epoxy as precolumn in flow through mode, followed by CIM® QA in load-elute mode.

> Figure 2
Process version 1 - Purification on CIM® QA

EQUILIBRATION LOAD ON CIM QA STEP ELUTION REGENERATION
Buffer A Load volume: 300 mL 10-30-50-100 % B 2 M NaCl, 1 M NaOH

Sf9 supernatant was loaded on CIM® QA directly after 0,8µm filtration. The corresponding chromatogram is seen in Figure 3.

> Figure 3
Chromatogram process version 1 – Purification on CIM® QA

Baseline separation of four peaks is achieved by well optimized step gradient elution. 15 fold concentrated, infective baculovirus elutes at 30 % B (440 mM NaCl, cond= 43,5 mS/cm) in peak 2. Elution fractions of each peak were pooled and analysed against active virus, total DNA and total protein content (Table 1). Total DNA as well as total protein analysis includes baculovirus DNA and protein as well.

> Table 1
Mass balance of process version 1 – CIM® QA

<table>
<thead>
<tr>
<th></th>
<th>Volume (mL)</th>
<th>Virus titer (pfu/mL)</th>
<th>Recovery active virus (%)</th>
<th>Total DNA (%)</th>
<th>Total protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load</td>
<td>300</td>
<td>3,25*10⁸</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Pool 1</td>
<td>14</td>
<td>7,25*10⁸</td>
<td>0,1</td>
<td>0,3</td>
<td>4,4</td>
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<tr>
<td>Pool 2</td>
<td>15</td>
<td>4,75*10⁸</td>
<td>68,2</td>
<td>48,5</td>
<td>8</td>
</tr>
<tr>
<td>Pool 3</td>
<td>15</td>
<td>3,75*10⁸</td>
<td>5,8</td>
<td>31,5</td>
<td>na</td>
</tr>
<tr>
<td>Pool 4</td>
<td>14</td>
<td>2,45*10⁸</td>
<td>3,5</td>
<td>26,9</td>
<td>na</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td></td>
<td>77,6</td>
<td>107,2</td>
<td>12,4</td>
</tr>
<tr>
<td>FT</td>
<td>300</td>
<td>0</td>
<td>0</td>
<td>5,8</td>
<td>67</td>
</tr>
</tbody>
</table>

Recovery of 68,2 % infective virus was achieved by this direct loading, single column purification protocol.

Considerations towards a further increase of virus recovery led to the introduction of CIM® Epoxy as precolumn.
Sf9 supernatant was 0.8 µm filtered and loaded on CIM® Epoxy in flow through mode before binding on CIM® QA. In order to avoid contamination of the elution fraction, the CIM® Epoxy precolumn was removed before applying the step gradient and elution was performed on CIM® QA only. The corresponding chromatogram is seen in Figure 5.

By capturing interfering compounds such as hydrophobic substances (lipids), the virus recovery in the main virus peak (peak 2) was increased to 87 % infective virus at a 18 fold concentration. Elution fractions of each peak were pooled and analysed against active virus, total DNA and total protein content (Table 2). Total DNA as well as total protein analysis includes baculovirus DNA and protein as well.
Conclusions:

A robust, scaleable and efficient purification scheme of infective baculovirus based on CIM® monoliths was developed. A process version featuring direct load of filtered cell culture supernatant on a single column or optional use of a monolithic precolumn was designed. Recoveries of active virus of 68 % and 87 % and concentration factors of 15 and 18, respectively were achieved.

More details can be found in the following article: