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Application Note

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In-Process Control of pDNA Production on CIMac™ pDNA Analytical Column

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Abstract

As the demand for plasmid DNA (pDNA) based gene therapy and vaccines increases, large-scale, cost-effective, and reproducible pDNA production is required. The key to success is a real-time in-process control method that ensures a high percentage of supercoiled pDNA in the final product. CIMac™ pDNA Analytical Column allows the monitoring of degradation products (open circular and linear pDNA), the removal of impurities (RNA), and ensures that each production step yields the amount of supercoiled pDNA anticipated.

Introduction

This application note is an example of a pDNA purification process (Table 1) based on our CIM® HIP² Plasmid Process Pack™ with in-process control steps shown in Figure 1. The final product composition is confirmed by Agarose Gel Electrophoresis in Figure 2. In Figure 3, the complete separation of all three pDNA conformations from a test solution within 10 minutes on the CIMac™ pDNA Analytical Column shows the versatility of the column.

Materials

CIM® HIP² Plasmid Process Pack™, CIMac™ pDNA Analytical Column

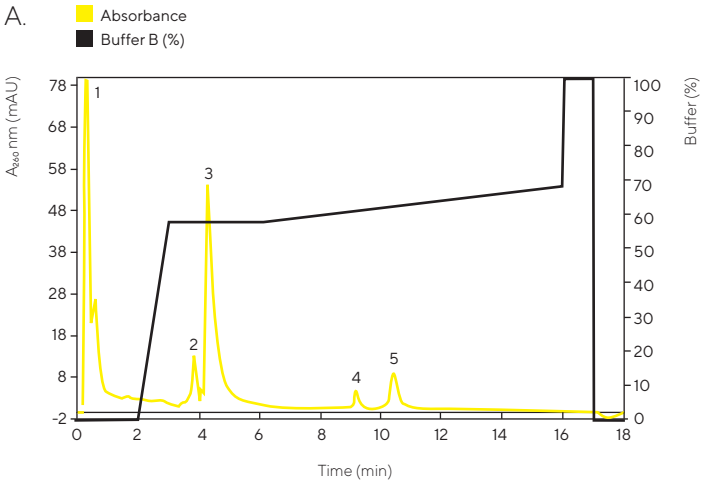
Methods

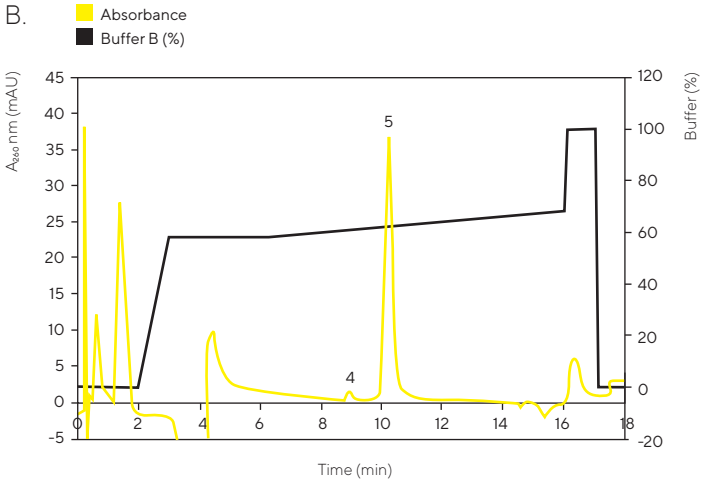
Usage of CIMac™ pDNA Analytical Column for in-process control

Column: CIMac™ pDNA Analytical Column (5.2 mm I.D. × 15.0 mm)

Injection volume	20 µL
Mobile phase A	Buffer A: 200 mM Tris, pH 8.0
Mobile phase B	Buffer B: 200 mM Tris, 1 M NaCl, pH 8.0
Detection	UV at 260 nm
Flow rate	1 mL/min
HPLC system	A high pressure gradient HPLC system, Agilent 1200

Sample: Alkaline lysate of plasmid pEGFP-N1 (4.7 kbp) after adjustment to 0.5 - 1.0 M $CaCl_2$ (A.) was diluted 1:3 with water and filtered 0.45 µm prior analysis. Eluate of CIM DEAE (B.) was diluted 1:3 with water, whereas eluate of CIM C4 (C.) was directly injected onto CIMac™ pDNA Analytical Column.





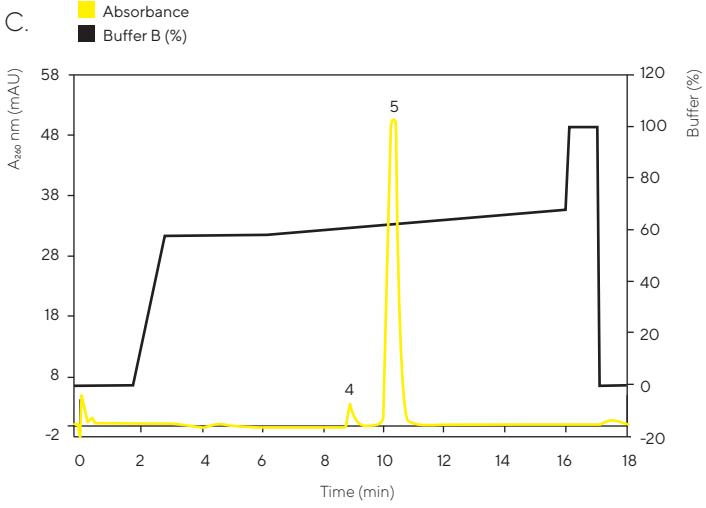
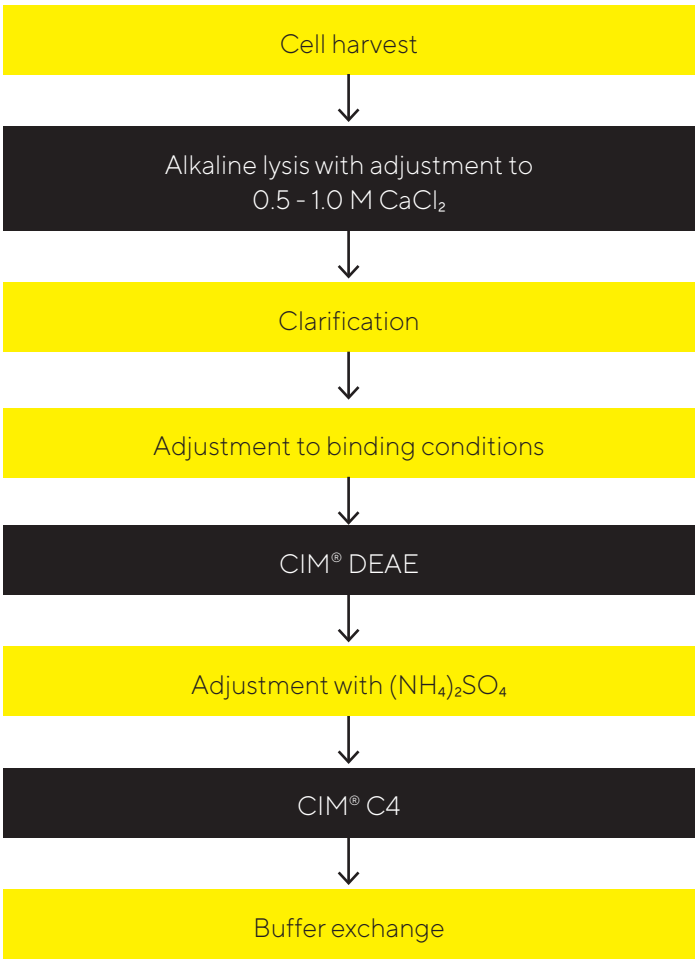


Figure 1: Usage of CIMac™ pDNA Analytical Column for in-process control after alkaline lysis with adjustment to $CaCl_2$ (A.), capture step (B.) and polishing step (C.)

Table 1: Purification scheme of pDNA Purification with CIM® HIP² Plasmid Process Pack™



Confirmation by agarose gel electrophoresis
Molecular weight marker (lane M), sample alkaline lysate plasmid pEGFP-N1 (4.7 kbp, lane A), peak 1 (lane 1), peak 2 (lane 2), peak 3 - RNA (lane 3), peak 4 - pDNA open circular (lane 4), peak 5 - pDNA supercoiled (lane 5), pDNA open circular standard (lane 6).

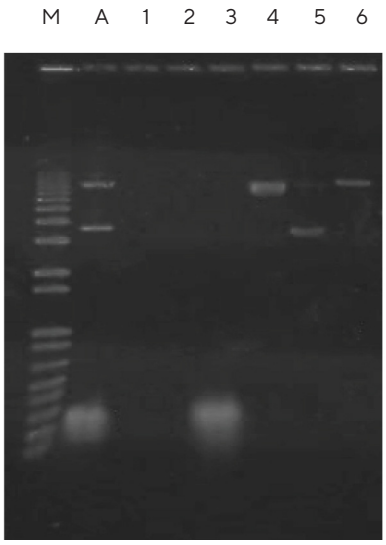


Figure 2: Confirmation by agarose gel electrophoresis

Efficient separation of all three conformations
Column: CIMac™ pDNA Analytical Column (5.2 mm I.D. × 15.0 mm)

Injection volume	10 µL
Mobile phase A	Buffer A: 200 mM Tris + 0.6 M NaCl, pH 8.0
Mobile phase B	Buffer B: 200 mM Tris + 0.7 M NaCl, pH 8.0
Gradient	a linear gradient from 0 to 100 % buffer B in 10 min
Detection	UV at 260 nm
Flow rate	1 mL/min
HPLC system	A high pressure gradient HPLC system, Agilent 1200

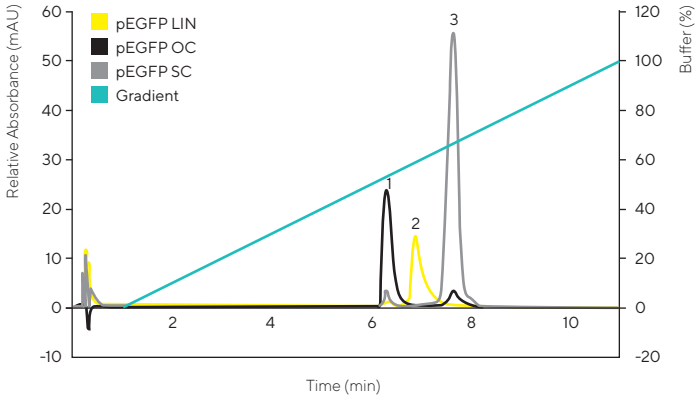


Figure 3: Efficient separation of all three conformations

Results

When used as part of a complete production process, each in-process control check is completed in less than 18 minutes (Figure 1) enabling the comparison of real time data during the production of your gene therapy or vaccine product; saving time and expensive wastage. Additionally, CIMac™ pDNA Analytical Column enables the separation of all three pDNA conformations (Figure 3) and is able to monitor the removal of other impurities such as RNA.

Conclusion

CIMac™ pDNA Analytical Column allows quality control to ensure that each batch has the highest level of supercoiled pDNA and the minimum amount of impurities in real time to ensure an efficient and cost effective gene therapy | vaccine production run.

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