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Application Note

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Recovery of pDIKE2 Plasmid for Hepatitis C Vaccine Using CIMmultus® C4 HLD Monolith

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Abstract

pDNA plays a crucial role in modern healthcare, specifically in the gene therapy field. Its main application is as the delivery vehicle, or vector, to introduce foreign DNA into organisms. Monolithic columns can be used in the production of pDNA as raw material or therapeutic products. This application note shows the results of pDNA purification with the CIMmultus® C4 HLD column.

Introduction

DNA immunization can potentially induce both humoral and cellular immune responses, making it an attractive approach to developing an effective vaccine against HCV. The pIDKE2 plasmid is the main component of the CIGB's candidate vaccine against Hepatitis C virus (HVC), which is used to treat HCV chronically-infected individuals during clinical trials phase 1 and 2.

An improvement to the downstream process was required to produce the necessary quantities to meet the high demand for plasmids for clinical trials.

Materials

CIMmultus® C4 HLD 800 mL cGMP Compliant Monolithic Column (HLD Butyl)

Methods

After size exclusion chromatography the pDNA fraction eluted was applied on CIMmultus® C4 HLD-800 Advanced Composite Column cGMP, finally the elution was concentrated until 2 mg/mL and filtered at 0.2 µm.

Column: CIMmultus® C4 HLD 800 mL cGMP Compliant Monolithic Column (HLD Butyl)

Mobile phases	Buffer A: 1.75 M $(NH_4)_2SO_4$, 0.025 M Tris; 0.010 EDTA pH7 and Buffer B: 0.025 M Tris; 0.010 EDTA pH7
Flow rate	300 mL/min
Equlibration of column	100% Buffer A, washing after sample loading: 100% Buffer A, elution: 100 % Buffer B
Load	2.6 L
Detection	λ = 254 nm
System	Sepacore System from Buchi

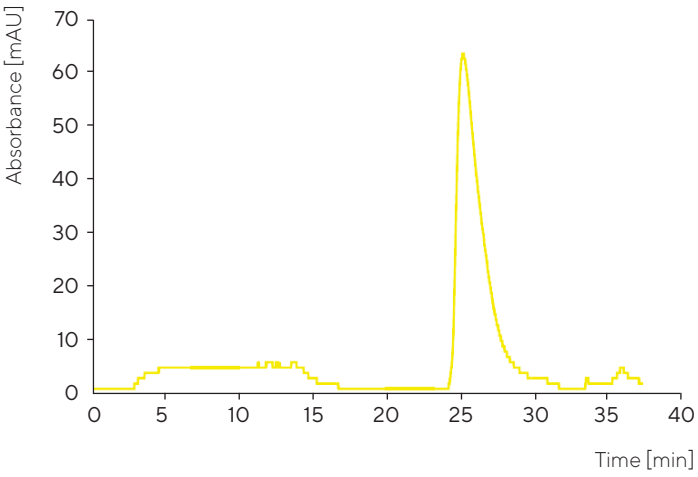
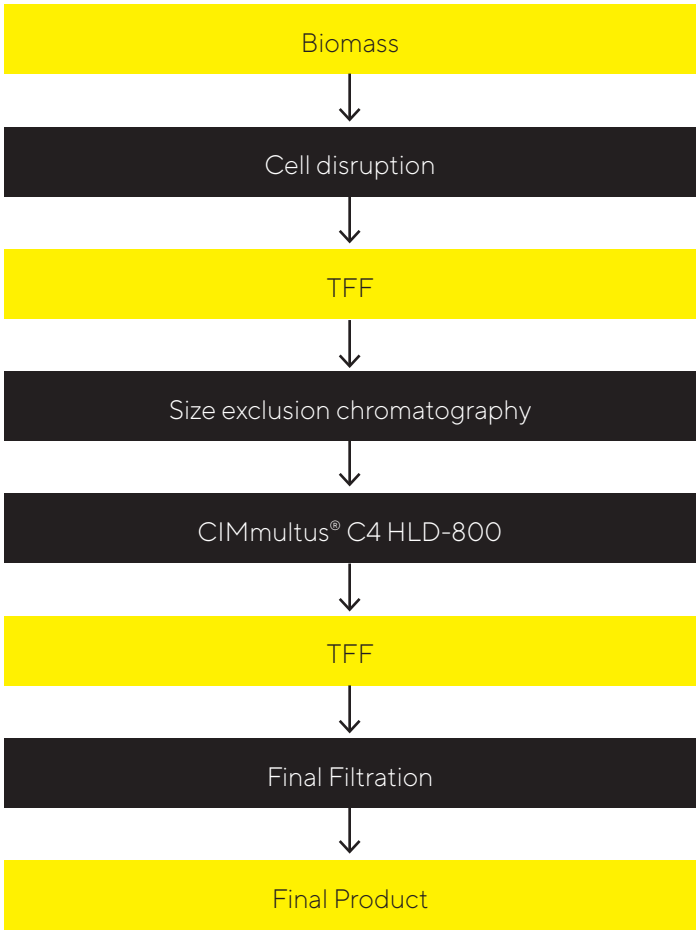


Figure 1: Chromatographic behavior of CIMmultus® C4 HLD 800 mL column, cGMP

Purification Scheme



Column: CIMac™ pDNA-0.3 Analytical Column

Mobile phases	Buffer A: Buffer A: 200 mM Tris + 0.6 M NaCl, pH 8.0 and Buffer B: 200 mM Tris + 0.7 M NaCl, pH 8.0
Flow rate	1 mL/min
Step gradient method	A linear gradient from 0 to 100% Buffer B in 10 min.
Load	2.6 L
Detection	λ = 254 nm
System	HPLC Merck - Hitachi

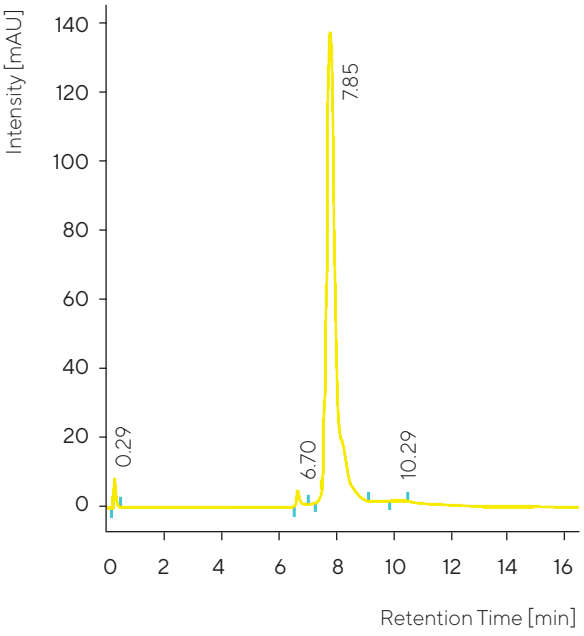


Figure 2: Elution of CIMmultus® C4 HLD fraction analyzed on CIMac™ pDNA-0.3 Analytical Column

Results

Purified pDNA contains more than 95% purity of pIDKE2. The content of genomic DNA is lower than 5 µg per dose, RNA is not detectable by agarose gel electrophoresis; endotoxin content is below 5.0 EU per kg body weight, and the protein content is 1.4 µg per dose, which is lower than the limit established.

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