SARTORIUS

Application Note

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Quick Start Methods for pDNA Isoform Monitoring With CIMac pDNA

Baseline Separation of Key pDNA Isoforms

Correspondence
Email: help@biaseparations.com

Abstract

CIMac pDNA Analytical Column is powerful tool for pDNA quantification for in-process control or in a QC laboratory. The column separates pDNA isoforms from each-other and from RNA impurities. Monitoring of pDNA production leads to a controlled and robust process, and can result in consistent high quality of the final product.

Optimised methods are a key component of a well-functioning analytical system, sometimes requiring time-consuming method development and steep learning curves. The following two methods described in this quick start guide can provide a starting point for pDNA purity and isoform analysis.

Figure 1: *NaCl Gradient*

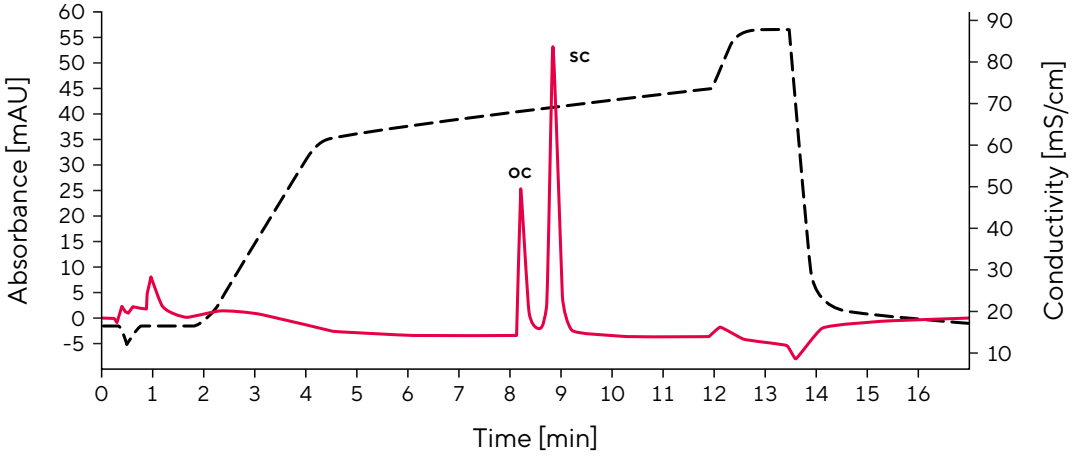


Figure 2: *Method B – NaCl Gradient With Addition of Guanidine (Gnd) Hydrochloride*

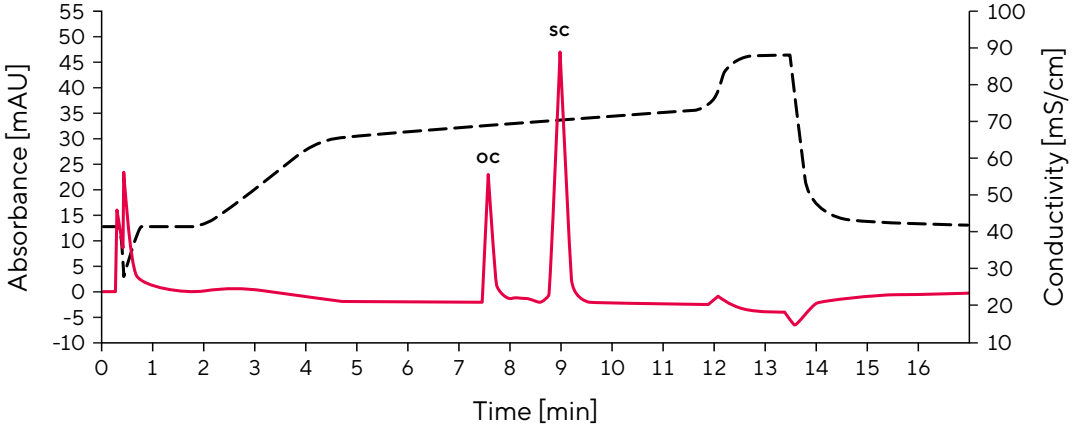


Table 1: *Quick Start Methods With and Without Guanidine for pDNA Isoform Analytics*

Method name	**NaCl (see figure 1)**		**NaCl gradient with addition of guanidine (Gnd) hydrochloride (see figure 2)**	
Equilibration buffer	100 mM Tris, pH 7.9		100 mM Tris, 0.3 M Gdn, 0.1 M NaCl, pH 8.0	
Elution buffer	100 mM Tris, 1 M NaCl, pH 7.9		100 mM Tris, 0.3 M Gdn, 0.7 M NaCl, pH 8.0	
Flow	1 mL/min		1 mL/min	
System	PATfix® pDNA analytical platform		PATfix® pDNA analytical platform	
Detection	UV 260 nm		UV 260 nm	
Sample	pFix5 standard (5 kbp, Catalog no. BIA-pFix5.1.1)		pFix5 standard (5 kbp, Catalog no. BIA-pFix5.1.1)	
Method gradient	**Time (min)**	**Elution buffer (%)**	**Time (min)**	**Elution buffer (%)**
	0 min	10%	0 min	0%
	1 min	10%	1 min	0%
	3 min	66%	3 min	50%
	11 min	81%	11 min	68%
	11 min	100%	11 min	100%
	12.5 min	100%	12,5 min	100%
	12.5 min	10%	12,5 min	0%
	17 min	10%	17 min	0%

Multimer and Linear pDNA Analysis

CIMac pDNA enables separation and quantification of multimer pDNA and linear pDNA isoforms.

Figure 3: *Multimer Isoform(s) of pFix5 Present in the Sample Can be Detected Using the Improved Method With Additonal Guanidine*

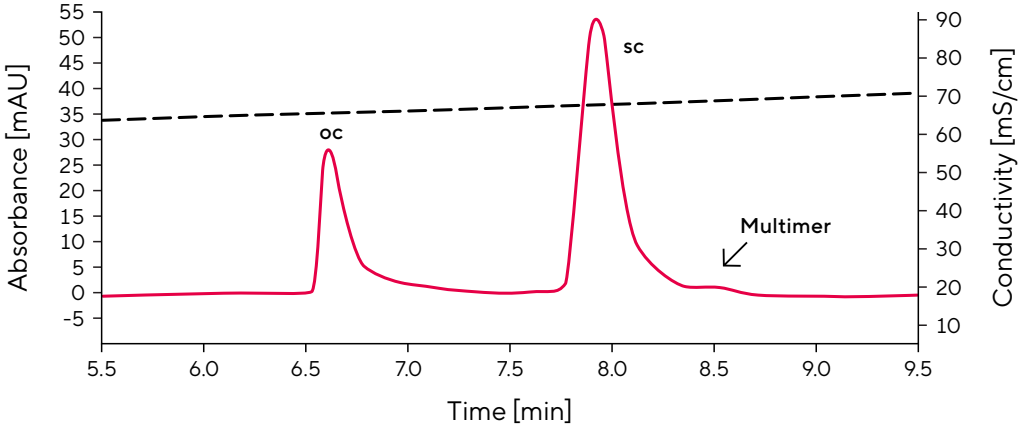
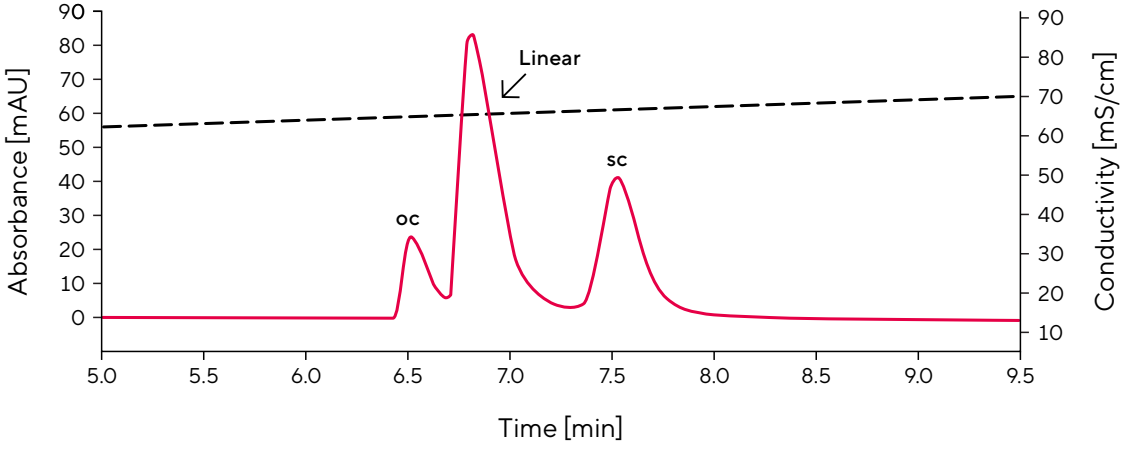


Figure 4: *Separation of Relevant Isoforms for pDNA Linearization Monitoring*



Elution with a sodium chloride gradient while maintaining guanidine hydrochloride at a level concentration of 300 mM achieves close to 100% recovery, preventing carry-over, and can lead to improved resolution between the isoforms. In addition to oc and sc isoform peaks, linear and multimer pDNA are also visible.

Notice

- Results shown here were obtained using CIMac pDNA column with 1.4 µm diameter channels. For plasmids larger than ~7 kbp the analytical column with 6 µm channel diameter should be used. Larger channels limit the entrapment of oc isoform within the monolith and improve quantification. The methods may need optimisation with CIMac pDNA 6 µm. to avoid decreasing pDNA oc isoform recovery due to steric constraints.
- For optimal performance, it is best that samples are in the same or very similar buffer that is used at the beginning of the analytical method. That is usually the equilibration buffer.
- The recommended starting procedures for sample preparation of different fractions is listed in the table below.

Sample	Recommended sample prep & dilutions*
Alkaline lysate	centrifugation + 50x dilution
Neutralized lysate	centrifugation + 50x dilution
Lysate after coarse filtration	centrifugation + 50x dilution
Lysate after fine filtration	50x dilution
DEAE load, flow through, wash	10x dilution
DEAE elution	depending on pDNA concentration
CIP fraction	Neutralization and dilution
C4 HLD load, flow through, wash	at least 4x dilution, higher if possible
C4 HLD elution	at least 4x dilution, higher if possible

* For best results dilution should be performed with the equilibration buffer.

- Experiments were conducted using PATfix® pDNA analytical platform. For optimal results and method optimisation, the use of conductivity detector is highly recommended.
- Conductivity of the sample to be analysed must not be greater than the conductivity of method starting condition (40 ± 2 mS/cm), for the optimized method with included guanidine hydrochloride.
- All the results were obtained with guanidine hydrochloride of greater than 99% purity. Guanidine hydrochloride of lesser purity exhibited unwanted signals on UV baseline.
- Column temperature should be held constant using a column thermostat.
- The methods shown in our publications are intended as starting point for analytical work. It is recommended to conduct method optimisation to suit individual samples and HPLC systems.
- For maximum method robustness, a blank run should be added between each sample run.

Additional Literature

Černigoj U., Vidič J., Ferjančič A., Sinur U., Božič K., Mencin N., Martinčič Celjar A., Gagnon P., Štrancar A. 2021. Guanidine improves DEAE anion exchange-based analytical separation of plasmid DNA. Electrophoresis 0 (2021) 1–7

Technical note: CIMac pDNA-0.3 Analytical Column – Optimising Its Chromatographic Reproducibility, 2019

Application note: PATfix® pDNA analytics for in process control of linear pDNA production, 2021

Brochure: pDNA Downstream Processing Using CIM® Monoliths, 2019

Brochure: PATfix® pDNA analytical platform

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

Slovenia
Sartorius BIA Separations d.o.o.
Mirce 21
5270 Ajdovščina
Phone +386 59 699 500

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
www.sartorius.com

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