Purification of Plasmid DNA (pDNA) with AXISFLOW™ QA IEX COLUMNS

ABSTRACT

Plasmid DNA (pDNA) is a critical upstream component for various applications ranging from gene and cell therapy, mRNA platforms, and DNA vaccines. pDNA used in these applications must be highly pure and homogeneous. With surging demands of gene-based applications, the need for efficient large-scale production and purification of pDNA is more crucial than ever. This application note demonstrates the performance of AXISFLOW™[1] QA IEX column for purification of three different types of plasmids: small-sized (pUC19 - 2,686 bp), mid-sized (pRep2Cap9 – 6,821 bp) and large-sized (pHelper – 12,261 bp), purified from crude *E. coli* lysates. Furthermore, this note includes practical guidance on optimizing sample preparation and chromatographic parameters to maximize plasmid yield and purity.

INTRODUCTION

Plasmids are large molecules, which present significant challenges when purified using conventional chromatography media. These traditional materials suffer from low capacity[2] and throughput. For this reason, monoliths and membranes[3] are often the preferred materials due to their wide pore and channel sizes. Polymethacrylate-based monoliths, functionalized as anion exchangers, have been established as a standard for plasmid purification[4] and are already used at industrial scale[5,6]. However, there remains a demand for improved process performance.

The AXISFLOW™ technology represents a new category of chromatography media. Its unique, inverted flow morphology is characterized by a high void volume, up to 80%[7,8], which is generated from hollow spheres interconnected by large pores. This feature not only provides exceptional yields in plasmid purification but also maintains the mechanical stability required to operate at high flow rates. Furthermore, the high permeability of the chromatography media maintains low column pressure even during sample loading.

RESULTS AND DISCUSSION

Crude *E. coli* lysates containing pUC19, pRep2Cap9, or pHelper were clarified, filtered through a 0.22 µm filter, and buffer-exchanged into the equilibration buffer by dialysis or diafiltration before being loaded onto the AXISFLOW™ QA column. Post loading, the column was washed with equilibration buffer until the 260 nm absorbance signal returned to baseline, before starting a stepwise gradient elution of increasing salt concentration up to 1 M NaCl (Table 1). The 260 nm absorbance was monitored throughout the run. pDNA elutes at a conductivity of ≥ 52 mS/cm for the pDNA tested whereas impurities were eluted at a conductivity of ≤ 50 mS/cm (Figure 1 — data for diafiltration shown).

Table 1: Chromatographic methods for purification of plasmids by AXISFLOW™ QA anion exchange chromatography.

Phase	Buffer / Sample	Flow rate (CV/min)	Column Volume (CV)
Equilibration	A: 50 mM Tris, 10 mM EDTA, 0.1 M NaCl, pH 7.2	2	6
Sample Application	pDNA crude lysate	2	-
Wash	50 mM Tris, 10 mM EDTA, 0.1 M NaCl, pH 7.2	2	10
Elution (Stepwise Gradient)	A: 50 mM Tris, 10 mM EDTA, 0.1 M NaCl, pH 7.2 B: 50 mM Tris, 10 mM EDTA, 1.0 M NaCl, pH 7.2	2	Increase 10% B per step (10 CV each) from 20 – 100% B

Cleaning	2 M NaCl, 1 M NaOH	2	4
Neutralization	Water or equilibration buffer	2	10
Storage	20% ethanol	2	10

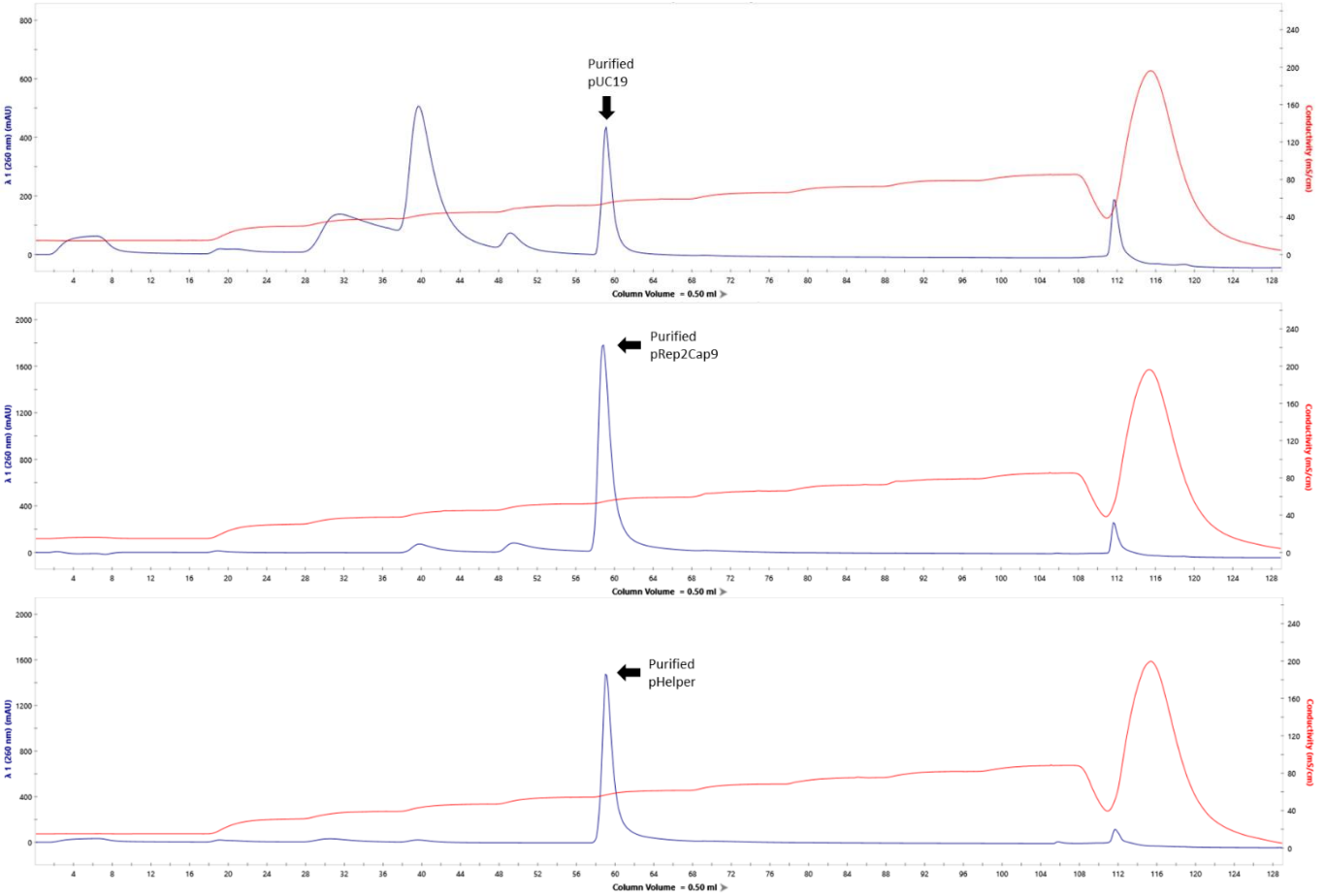

Figure 1: Chromatograms of various crude lysate (pre-treated by diafiltration) purified using AXISFLOW™ QA column. From top to bottom: (1) pUC19, (2) pRep2Cap9, (3) pHelper.

All samples purified with AXISFLOW™ QA consistently achieved high pDNA yields, regardless of plasmid size or the buffer-exchange method used during sample pre-treatment. Yields were over 90% for crude lysates pre-treated by dialysis, and over 80% for those pre-treated by diafiltration (Table 2). Analysis by anion-exchange HPLC confirmed that all purified plasmids had a supercoiled content of at least 90% and met the A260/A280 ratios between 1.80 and 2.00, meeting the minimum acceptance criteria for QC testing of high quality GMP-grade plasmids in cGMP manufacturing (Table 2).

Furthermore, the yield of the purified plasmid remained independent of flow rates, with consistent performance observed from 1 to 20 CV/min (Figure 2A). A consistent baseline separation was maintained, with no shift in retention time or change in peak width (Figure 2B - pRep2Cap9).

Table 2: Comparison of purity and quality of purified plasmids

Crude lysate pretreatment & plasmid	Column	Volume (ml)	Purified Eluate (supercoiled plasmid)			Achieved specifications for GMP grade
			% SC content	Purity (A260/A280)	% Yield	
pUC19 dialyzed	AXISFLOW™ QA	0.5	100%	1.90	92.1%	Yes
pUC19 diafiltered	AXISFLOW™ QA	0.5	100%	1.83	96.8%	Yes
pRep2Cap9 dialyzed	AXISFLOW™ QA	0.5	91.4%	1.88	95.7%	Yes
pRep2Cap9 diafiltered	AXISFLOW™ QA	0.5	92.4%	1.96	80.7%	Yes
pHelper dialyzed	AXISFLOW™ QA	0.5	90.9%	1.94	98.6%	Yes

pHelper diafiltered	AXISFLOW™ QA	0.5	90.6%	1.97	87.8%	Yes

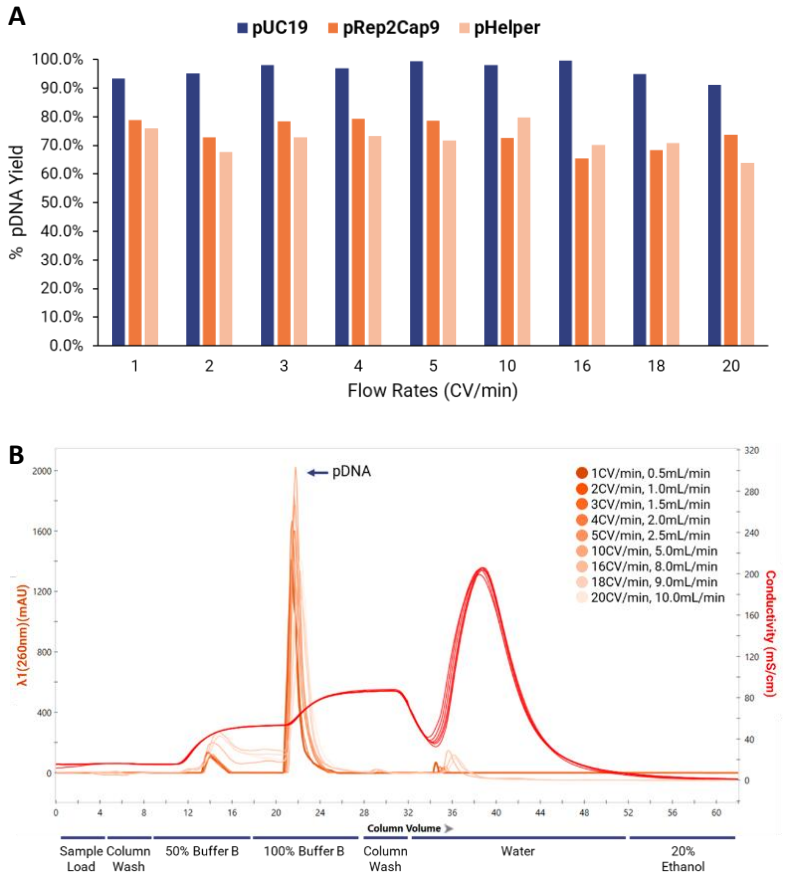

Figure 2: Flow-independent Performance of AXISFLOW™ QA (0.5 mL). (A) *% pDNA yield purified at different flow rates. (B) Overlay of chromatograms for pRep2Cap9 purification runs at different flow rates, ranging from 1-20 CV/min.*

SAMPLE PREPARATION

Crude *E. coli* lysates containing pUC19, pRep2Cap9, and pHelper plasmids were prepared using a standard alkaline lysis procedure[9]. After neutralization and clarification, the lysates containing high salt concentrations require buffer exchange to lower the ionic strength prior to loading onto the AXISFLOW™ QA column. This can be accomplished by dialysis or diafiltration into the equilibration buffer. The recommended equilibration buffer (Table 1) has a conductivity of 15 mS/cm. In some cases, higher conductivities—up to 30 mS/cm—have been shown to improve plasmid purity. Prior to column loading, the pDNA sample should be pre-filtered through a 0.22-µm filter to remove particulates or precipitates to prevent column clogging.

ELUTION GRADIENT CONSIDERATIONS

A stepwise or multistep gradient elution is recommended when purifying a plasmid for the first time or when working with samples that contain high levels of impurities. Continuous gradient elution can also be effective, particularly when using a higher starting ionic strength. The optimal ionic strength should be determined empirically for each plasmid and sample type.

CONCLUSION

This application note demonstrates the robustness of the AXISFLOW™ QA column in isolating plasmids from crude lysate, using different pre-treatment methods. Our crude lysate preparations did not require RNase treatment, simplifying the process. AXISFLOW™ QA consistently delivered high yields and high-resolution separations for plasmids ranging from 2,686 to 12,261 base pairs. Notably, these results were robust and reproducible even at high flow rates of up to 20 CV/min. This highlights the column's suitability for high-speed, large-volume manufacturing environments.

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