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Application Note

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Plasmid DNA Purification

Ion Exchange Chromatography With Sartobind® Membrane Adsorbers

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Introduction

Plasmid DNA (pDNA) is produced in E. coli and some eukaryotic organisms. The circular and double stranded pDNA carries extra-chromosomal information and can replicate independently. Plasmids must be purified properly to transform a bacterial genome or when used as a vector for patient cells.

Sartobind® Q anion exchange membranes with >3 µm pore size are useful to bind negatively charged plasmids without size exclusion effects. Contaminating RNA can pass through as the pDNA charge is higher and separation is possible by salt addition at equilibration.

In the following pair of experiments two different flow and conductivity conditions have been applied to recover 150 mg pDNA.

Materials

- Liquid chromatography system
- Sartobind® Q SingleSep 5" capsule (92IEXQ42D9-SS), ligand: quaternary ammonium, bed height: 4 mm, bed volume: 70 mL
  (The successor of the capsule is Sartobind® Q 75 mL, 4 mm, 96IEXQ42D9MFF--A, see foto)

Plasmids

- 5-6 kb from E. coli production and cell lysis, lysate volume: 8.7 L, OD_{600} = 13
- Initially estimated quantity of pDNA: 150 mg

Buffers

- Equilibration | loading | wash buffer: 10 mM Tris + 0.1 M EDTA + 0.5 (trial 1) or 0.6 M NaCl (trial 2), pH 8
- Elution buffer: 10 mM Tris + 0.1 M EDTA + 1.2 M NaCl, pH 8

Methods

Plasmid purification

1. Preparation of the plasmid solution (E. coli fermentation, cell harvest and lysis)
2. Prefiltration with Sartopure® PP2 20 µm followed by Sartopore® 2 0.45 + 0.2 µm filter
3. Equilibration of Sartobind® Q device
4. Loading of plasmid onto Sartobind® Q device
5. Washing with equilibration buffer
6. Elution
7. Hydrophobic Interaction Chromatography for plasmid isoform separation (not described here)

Analysis

Electrophoresis

- on lysis solution
- on flow-through of Sartobind®
- on elution peak of Sartobind®
- after gel filtration
- after affinity column

Plasmid quantity estimation

- in lysis solution
- in eluted sample by Sartobind®
- after gel filtration
- after affinity column
- total throughput

Results

Trial 1

Equilibration	Buffer with 0.5 M NaCl
Flow rate	200 mL/min
Operation pressure	approx. 0.02 MPa (0.2 bar, 3 psi)
Equilibration volume	1.9 L
Load volume	3.5 L
Elution volume	1.1 L
Second elution	3 M NaCl to elute all nucleic acids

Electrophoresis

Elution at 1.2 M NaCl	RNA traces
Elution at 3 M NaCl	rest of DNA, no RNA

Yield

43 mg pDNA

Summary trial 1

Elution peaks: good, just minor dilution effect.

Trial 2

Equilibration	Buffer with 0.6 M NaCl
Flow rate	500 mL/min
Operation pressure	approx. 0.06 MPa (0.6 bar, 9 psi)
Equilibration volume	2.5 L
Load volume	4.1 L
Second elution	3 M NaCl to elute all nucleic acids

Electrophoresis

Elution at 1.2 M NaCl	no or few RNA traces
Elution at 3 M NaCl	recovery of whole DNA

Yield

78 mg pDNA

Summary trial 2

Only minor traces of RNA could be detected.

Summary

Total recovery of pDNA quantity was 43 + 78 = 121 mg resulting in 81% (initial pDNA quantity: 150 mg). The analysis displayed the presence of RNA but in small quantities. In trial 2 the use of 0.6 M NaCl buffer with a flow rate of 500 mL/min resulted in higher recovery and less binding of RNA.

Conclusion

Sartobind® Q membrane adsorber is a valuable tool for fast and high quality purification of pDNA.

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