PlasmidSelect
Supercoiled Plasmid Purification Starter Pack

PlasmidSelect is a thiophilic aromatic adsorption chromatography medium used as a selective capture step in a three-step chromatographic process for purifying supercoiled plasmid DNA. The operational and performance characteristics of the media and process are suitable for large-scale production of plasmid DNA.

- Selectively purifies supercoiled form of plasmid DNA
- Does not use components that require time-consuming removal or handling
- Scalable, RNase-free process for easy transfer to GMP compliant production
- Reusable after Cleaning in Place with NaOH
- Easily automated on ÄKTA™explorer for evaluation, documentation and convenience

Supercoiled Plasmid Purification Starter Pack contains PlasmidSelect as the selective capture step to purify supercoiled plasmid DNA. The other two media in the Starter Pack are Sepharose™ 6 Fast Flow for RNA removal and SOURCE™ 30Q, for sample concentration and polishing removal.

Introduction

From pre-clinical studies to production of plasmid DNA products
The PlasmidSelect purification process saves time and money by allowing the convenient transfer of pre-clinical plasmid DNA purification studies to large-scale, GMP compliant production. The process combines reproducibility and scalability with purity levels that meet gene therapy standards. Moreover, the process is easily automated and cleaned and does not involve precipitating agents, detergents, organic solvents or other components that require time-consuming removal or handling.

The Supercoiled Plasmid Purification Starter Pack is useful for laboratory scale purification of approximately 10 mg supercoiled plasmid DNA and more. Amersham Biosciences Regulatory Support Files assist to meet the regulatory demands related to GMP compliant production. Typical results from non-GMP conditions are shown in Table 1 below.

Fig 1. PlasmidSelect and the Supercoiled Plasmid Purification Starter Pack.

Table 1. Typical results from non-GMP conditions.

Supercoiled plasmid DNA	>97%
Plasmid DNA	up to 850 µg/ml
Endotoxins	< 10 EU/mg
RNA	below fluorescence detection limit
Proteins	below BCA-assay detection limit
Genomic DNA	< 0.2% of total DNA

Amersham Biosciences

The PlasmidSelect purification process

The three-step purification process as shown in the flow scheme (Fig 2) does not require RNase enzyme, organic solvents, detergents, precipitants or animal-derived components. The chromatographic steps start with clarified bacterial lysate and are as follows:

Step 1: RNA removal and buffer exchange by group separation using Sepharose 6 Fast Flow

Step 2: Selective capture of the supercoiled form of plasmid DNA by thiophilic aromatic adsorption chromatography using PlasmidSelect

Step 3: Polishing and concentration by anion exchange chromatography using SOURCE 30Q.

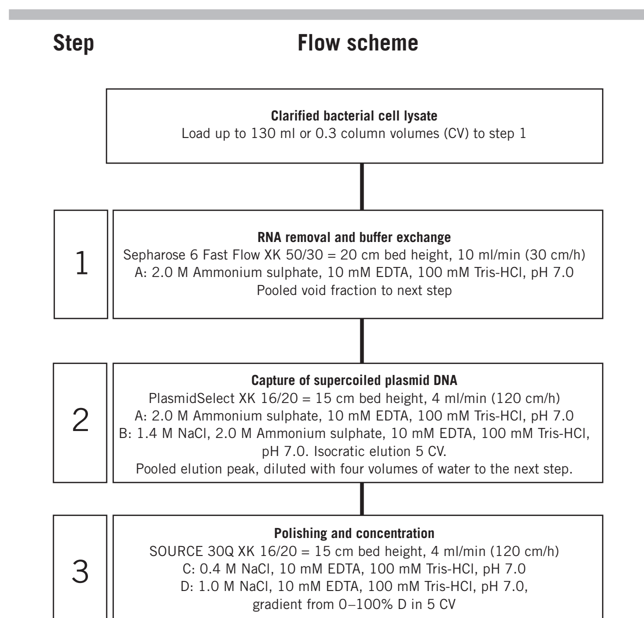

Fig 2. The process flow scheme.

The Supercoiled Plasmid Purification Starter Pack contains appropriate amounts of the three different media for purification of approximately 10 mg supercoiled plasmid DNA. Columns are not provided with the Starter Pack and have to be ordered separately. To purify plasmid DNA at other scales the flow velocity and other parameters can be used as guidelines for up- or downscaling. All Starter Pack media can be ordered separately in process scale quantities.

The basic characteristics of Sepharose 6 Fast Flow, PlasmidSelect and SOURCE 30Q are given below in Tables 3, 4 and 5. Detailed recommendations for method design and optimization, cleaning-sanitization and column packing are found in the instructions enclosed with Supercoiled Plasmid Purification Starter Pack or PlasmidSelect.

Application

Feed/sample preparation

The starting material for plasmid DNA preparation is usually clarified cell lysate prepared by alkaline lysis of bacteria or other cells. Protocols for preparation of cell lysates can be found in laboratory manuals such as (1).
An initial concentration of approximately 0.1 mg/ml plasmid DNA is recommended to obtain 10 mg supercoiled plasmid DNA in just one chromatography cycle. If a more diluted feed is used the first column should be run several times and all eluted void fractions containing the plasmid DNA should be loaded on the column containing PlasmidSelect.

If the initial concentration is below approximately 0.02 mg/ml e.g. with a low plasmid copy number, consider the use of Amersham Biosciences Cross Flow Membranes to concentrate the feed material for better process economy. We suggest the use of 300 000 NMWC ultrafiltration

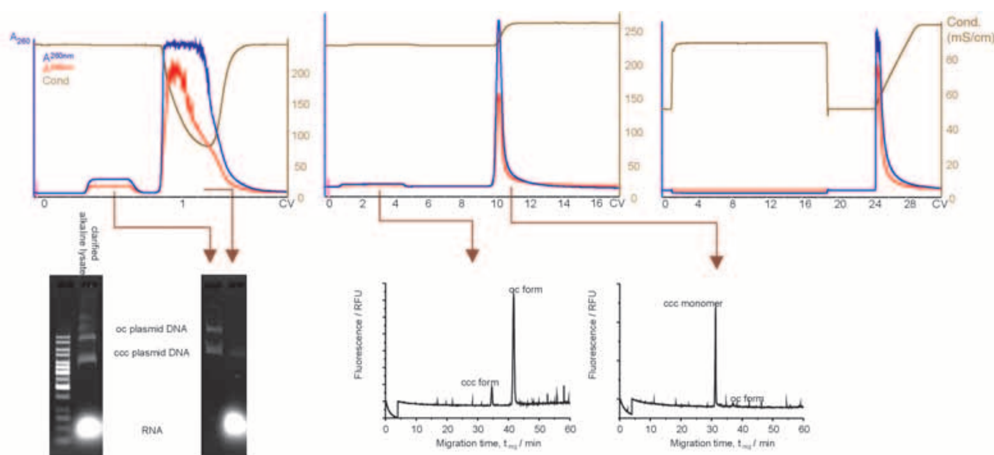

Fig 3. PlasmidSelect process for purification of supercoiled plasmid DNA, (Capillary gel electrophoresis performed by PlasmidFactory, Bielefeld, Germany).

membrane for this task. Ultrafiltration cartridge selection (i.e. chosing the most suitable membrane surface area) depends on the volume of the starting material and the process time objective. Cross Flow Ultrafiltration is linearly scalable.

A QuixStand™ BenchTop Laboratory Cross Flow System installed with a 300 000 NMWC membrane cartridge containing 140 cm^2 membrane surface area can be used for 10X concentration of 1200 ml in approximately 30 minutes. Contact Amersham Biosciences for assistance in matching feed volume to membrane surface area and to select the appropriate Ultrafiltration System.

An example of how high-purity supercoiled plasmid DNA is attained after following the three chromatographic steps is outlined in Figure 3.

Step 1 – RNA removal and buffer exchange with Sepharose 6 Fast Flow

RNA is removed from the clarified lysate by group separation.

Clarified alkaline lysate is first loaded in batches of 0.3 column volumes (CV) at 30 cm/h in an XK50/30 column packed to 20 cm with Sepharose 6 Fast Flow in order to change the buffer to 2.0 M $(NH_4)_2SO_4$, 10 mM EDTA, 100 mM Tris-HCl, pH 7.0. Simultaneously, this procedure also removes RNA and other contaminants (Ethidium Bromide stained 1% agarose gel electrophoresis picture below chromatogram).

Step 2 – capture of supercoiled plasmid DNA with PlasmidSelect

Supercoiled plasmid DNA is separated from open circular plasmid DNA and remaining contaminants such as residual genomic DNA and RNA.

The pooled void fraction (from Step 1) is subsequently applied at 120 cm/h on a XK 16 column packed to 15 cm bed height with PlasmidSelect and equilibrated in the same buffer as in Step 1. The column is washed and supercoiled plasmid DNA is eluted with 1.4 M NaCl, 2.0 M $(NH_4)_2SO_4$, 10 mM EDTA, 100 mM Tris-HCl, pH 7.0 (conductivity, brown). Open circular forms of plasmid are not retained by the column (inserts below chromatogram, electropherograms after laser induced fluorescence capillary gel electrophoresis (PlasmidFactory, Bielefeld, Germany)).

Step 3 – Polishing and concentration with SOURCE 30Q

Endotoxins are further removed and at the same time, the supercoiled plasmid DNA preparation is concentrated by ion exchange chromatography.

The peak containing supercoiled plasmid DNA is diluted with 4 volumes of water and loaded at 120 cm/h on a XK16/20 column with SOURCE 30Q packed to 15 cm bed height. The column is equilibrated in 0.4 M NaCl, 10 mM EDTA, 100 mM Tris-HCl, at pH 7.0 and eluted with a linear gradient by 1.0 M NaCl, 10 mM EDTA, 100 mM Tris-HCl, at pH 7.0.

Table 2. An example from a non-optimized fermentation of low-copy number plasmid (6.1 kb)*.

	Capillary gel electrophoresis		Picogreen	TaQMan-PCR	Ribogreen	BCA	LAL test
	ccc†	Open Circular	Plasmid DNA	Genomic DNA	RNA	Proteins	Endotoxins
	(%)	(%)	(µg/mL)	(µg/mg plasmid DNA)	(µg/mg plasmid DNA)	(µg/mg plasmid DNA)	(EU/mg plasmid DNA)
Clarified Lysate	54	46	64	150	203	60 000	150 000
Sepharose 6FF (void-peak)	71	29	52	6	< 100	1077	4800
Sepharose 6FF (RNA-peak)	–	–	3	126	35 000	530 000	65 000
PlasmidSelect (flow-through)	–	100	1	8	< 500‡	8 000	46 000
PlasmidSelect (plasmid DNA-peak)	96	4	128	6	< 4‡	109	470
SOURCE 30Q (plasmid DNA-peak)	97	3	326	2	< 0.25‡	< 3‡	9

* The analyses are performed by PlasmidFactory (www.PlasmidFactory.com).

† Covalently closed circle form of plasmid DNA.

‡ Below detection limit.

Results

Results from a low copy number case are shown in Table 2. 300 g of cell paste from a non-optimized fermentation gives about 9 liters of clarified lysate that finally results in 10 mg of purified supercoiled plasmid DNA.

PlasmidSelect

PlasmidSelect is the key protocol component since it interacts differentially with nucleic acids by thiophilic aromatic adsorption in the presence of water structuring salts (2) (Fig 4). This enables the topoisomere-selective purification of native supercoiled plasmid DNA and removal of damaged, nicked or open circular DNA by simple adjustment of chromatographic conditions. A group separation for removal of RNA prior to application on the column is strongly recommended. The capacity of PlasmidSelect for binding of the supercoiled form of plasmids will thus be fully utilized. Furthermore, group separation with Sepharose 6 Fast Flow greatly reduces the risk of precipitation during addition of ammonium sulfate and limits the variation in initial salt concentration that can influence selectivity, thus giving the process considerable robustness.

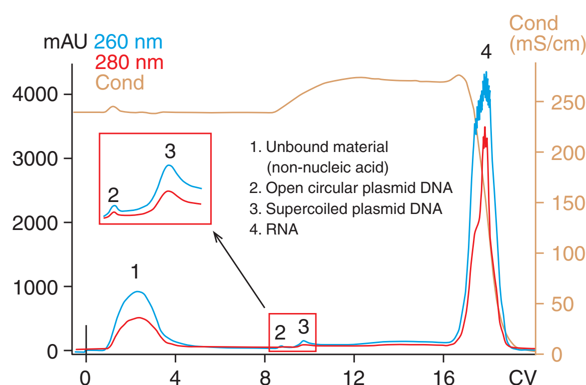

Fig 4. Demonstration of the selectivity of PlasmidSelect. Clarified alkaline lysate was prepared according to (3) and diluted with 1.1 volume of 4.0 M $(NH_4)_2SO_4$, 10 mM EDTA, 100 mM Tris-HCl, pH 7.0. The sample was loaded on a 4.6x150 mm column packed with PlasmidSelect. Elution was initiated with 3 M NaCl in 2.25 M $(NH_4)_2SO_4$, 10 mM EDTA, 100 mM Tris-HCl, pH 7.0 at 120 cm/h, and after 5 CV completed with a gradient to water. Open circular plasmid DNA is separated from supercoiled plasmid DNA in the salt gradient, while RNA and other contaminants such as proteins and endotoxins only elute in water.

Table 3. Characteristics of Sepharose 6 Fast Flow

Matrix	Highly cross-linked agarose 6%
Mean particle size	90 µm
pH stability	
Regular use	3–13
Cleaning	2–14
Cleaning-in-place	≤ 1 M NaOH
Linear flow velocity at 25 °C	
General	≤ 400 cm/h at 100 kPa (1 bar, 14.5 psi)
for supercoiled plasmid purification	≤ 30 cm/h, XK 50/30, 20 cm bed height
Operating temperature	4–40 °C
Storage temperature	4–30 °C
Delivery conditions	20% Ethanol

Table 4. Characteristics of PlasmidSelect.

Matrix	Highly cross-linked agarose 6%
Ligand	2-mercaptopyridine
Ligand concentration	4 mg/ml
Capacity for supercoiled pDNA (6125 bp)	> 0.4 mg/ml
Mean particle size	90 µm
pH stability	
Regular use	3–11
Cleaning	2–13
Cleaning-in-place	0.5 M NaOH
Linear flow rate at 25 °C	
General	≤ 400 cm/h at 100 kPa (1 bar, 14.5 psi)
for supercoiled plasmid purification	≤ 120 cm/h, XK 16/20, 15 cm bed height
Operating temperature	15–30 °C
Storage temperature	4–30 °C
Delivery conditions	20% Ethanol

Table 5. Characteristics of SOURCE 30Q

Matrix	Polystyrene/divinyl benzene
Type of ion exchange group	Quaternary ammonium
Mean particle size	30 µm
pH stability	
Working range	2–12
Cleaning	1–14
Linear flow velocity at 25 °C	
General	≤ 1000 cm/h
for supercoiled plasmid purification	≤ 120 cm/h, XK 16/20, 15 cm bed height
Cleaning-in-place	≤ 1.0 M NaOH
Operating temperature	4–40 °C
Storage temperature	4–30 °C
Delivery conditions	20% Ethanol

Ordering Information

Product	Pack size	Code No
PlasmidSelect	200 ml	17-5257-02
	1 liter	17-5257-03
	5 liter	17-5257-04
Supercoiled Plasmid Purification Starter Pack (contains 500 ml Sepharose 6 Fast Flow 30 ml Plasmid Select and 25 ml SOURCE 30Q)	1 pack	17-6002-94

Related Products

SOURCE 30Q	50 ml	17-1275-01
	500 ml	17-1275-02
	1 liter	17-1275-03
Sepharose 6 Fast Flow	1 liter	17-0159-01

Custom Packed Columns

Sepharose 6 Fast Flow XK 50/30	1	90-1003-68
PlasmidSelect, XK 16/20	1	90-1003-70
SOURCE 30Q, XK 16/20	1	90-1003-80

Lab-Scale Columns

XK 16/20 (16 mm i.d.)	18-8773-01
XK 50/30 (50 mm i.d.)	18-8751-01
Packing Connector XK 16	18-1153-44
RK50 reservoir	18-8790-01
XK 50 Fast Flow Kit	18-1000-70
Filter kit HR 16	18-3585-01

Ultrafiltration apparatus

1. Quix Stand BenchTop Laboratory Cross Flow system
   Model Number QSM–03SP
2 300 000 NMWC membrane cartridge
   Cartridge Model Number UFP–300–C–3MA

Handbook

Process Chromatography: A practical guide	18-1060-48

Data Files

SOURCE 30Q	18-1107-12
Sepharose 6 Fast Flow	18-1020-52
BPG™ 100, 140, 200, 300	18-1115-23
BPG 450	18-1060-59
Chromaflow™	18-1138-92
FineLINE™ Pilot 35	18-1104-95
FineLINE 100/100L, 200/200L	18-1130-00

Information about applications and suggested protocols for our products is available from our web site at http://bioprocess.amershambiosciences.com

References

1. Sambrook, J., and Russel, D. W. (2001) *Molecular cloning. A laboratory manual.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
2. Lemmens, R., Stadler, J., and Nyhammar, T. (2001) S-aryl ligands for chromatographic isolation of supercoiled plasmid DNA. *9th Meeting of the European Society of Gene Therapy, Antalya, Turkey* Nov. 2-4, 18-1157-1180
3. Horn, N. A., Meek, J. A., Budahazi, G., and Marquet, M. (1995) Cancer gene therapy using plasmid DNA: purification of DNA for human clinical trials. *Human Gene Therapy* 6, 565-573

to order:

Asia Pacific Tel: +852 2811 8693 Fax: +852 2811 5251 **Australasia** Tel: +61 2 9899 0999 Fax: +61 2 9899 7511 **Austria** Tel: 01 576 0616 20 Fax: 01 576 0616 27 **Belgium** Tel: 0800 73 888 Fax: 03 272 1637 **Canada** Tel: 1 800 463 5800 Fax: 1 800 567 1008 **Central, East, South East Europe** Tel: +43 1 982 3826 Fax: +43 1 985 8327 **Denmark** Tel: 45 16 2400 Fax: 45 16 2424 **Finland & Baltics** Tel: +358 (0)9 512 3940 Fax: +358 (0)9 512 1710 **France** Tel: 0169 35 67 00 Fax: 0169 41 9677 **Germany** Tel: 0761 4903 401 Fax: 0761 4903 405 **Italy** Tel: 02 27322 1 Fax: 02 27302 212 **Japan** Tel: 81 3 5331 9336 Fax: 81 3 5331 9370 **Latin America** Tel: +55 11 3667 5700 Fax: +55 11 3667 87 99 **Middle East and Africa** Tel: +30 (1) 96 00 687 Fax: +30 (1) 96 00 693 **Netherlands** Tel: 0165 580 410 Fax: 0165 580 401 **Norway** Tel: 2318 5800 Fax: 2318 6800 **Portugal** Tel: 21 417 7035 Fax: 21 417 3184 **Russia & other C.I.S. & N.I.S.** Tel: +7 (095) 232 0250,956 1137 Fax: +7 (095) 230 6377 **South East Asia** Tel: 60 3 8024 2080 Fax: 60 3 8024 2090 **Spain** Tel: 93 594 49 50 Fax: 93 594 49 55 **Sweden** Tel: +46 18 612 19 00 Fax: +46 18 612 19 10 **Switzerland** Tel: 01 802 81 50 Fax: 01 802 81 51 **UK** Tel: 0800 616 928 Fax: 0800 616 927 **USA** Tel: +1 800 526 3593 Fax: +1 877 295 8102

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