PlasmidSelect Xtra
PlasmidSelect Xtra Screening Kit
PlasmidSelect Xtra Starter Kit

PLASMID PURIFICATION MEDIA

Purified plasmid DNA is required in increasingly larger quantities to meet emerging requirements for gene therapy and DNA vaccination. As both human and veterinary applications are in focus, plasmid DNA purity, quantity, and cost-per-dose requirements vary greatly. Processes developed to purify plasmid DNA at industrial scale must thus be flexible, easily scalable, robust, and economical.

PlasmidSelect Xtra chromatography medium forms the basis of a generic process for purifying supercoiled (sc) covalently closed circular plasmid DNA suitable for bulk to clinical-grade applications. The process provides high capacity, delivers high yields, and can be scaled up to fulfill requirements for the economical industrial manufacture of plasmid DNA in highly regulated environments. The same principle can also be used to rapidly analyze the quantity and quality of plasmid DNA in complex solutions. Figure 1 shows a schematic illustration of the PlasmidSelect Xtra process.

PlasmidSelect Xtra platform

- Generic process for purification of supercoiled plasmid DNA
- Consistent from research to cGMP manufacturing
- Screening kit: Quick and easy analysis with an ÄKTA™ chromatography system
- Starter kit: Prepacked columns for convenient process development
- Bulk medium: PlasmidSelect Xtra medium is a BioProcess™ medium available in large quantities for scale-up and manufacture

From fermentation to formulation

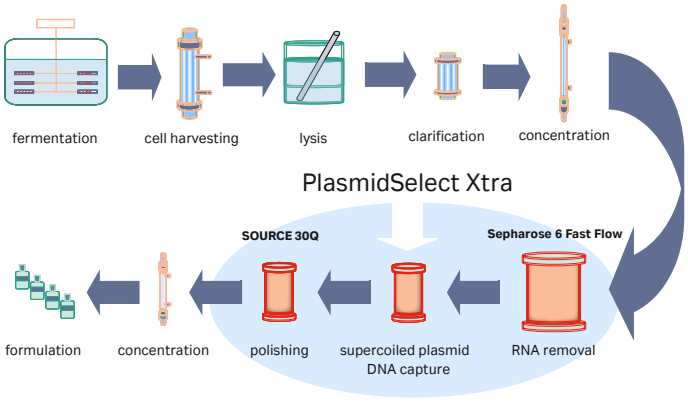

Fig 1. The PlasmidSelect Xtra process is designed for the purification of high-quality supercoiled plasmid DNA and for fast analysis of plasmid DNA quantity and quality. PlasmidSelect Xtra Starter Kit contains media in prepacked columns for each of the three chromatography steps. Samples can be withdrawn and analyzed using PlasmidSelect Xtra Screening Kit at any point from fermentation to RNA removal.

The PlasmidSelect Xtra process comprises:

1. RNA removal and buffer exchange by group separation on Sepharose™ 6 Fast Flow.
2. Capture and selective desorption of supercoiled plasmid DNA with PlasmidSelect Xtra.
3. Final polishing on SOURCE™ 30Q.

The complete purification process comprises both chromatography and filtration steps, such as clarification and ultrafiltration, before and after the three chromatography steps (Fig 1).

High-quality supercoiled plasmid DNA

The PlasmidSelect Xtra platform is designed to produce purified supercoiled DNA of high quality for gene therapy and DNA vaccination applications. Analyzing the purity of the final product is therefore a key aspect of the overall procedure. Table 1 (page 5) lists a detailed analysis of the purification of plasmid DNA purified with the PlasmidSelect Xtra process at different scales.

Three configurations

1. PlasmidSelect Xtra bulk medium – a thiophilic aromatic adsorption chromatography medium with a selectivity that allows supercoiled covalently closed circular forms of plasmid DNA to be separated from open circular forms.
2. PlasmidSelect Xtra Screening Kit – HiTrap™ Sepharose HP 5 ml and HiTrap PlasmidSelect Xtra 1 ml columns for quick and convenient determination of plasmid DNA quantity and quality during fermentation or purification process development (Figs 2 and 3).
3. PlasmidSelect Xtra Starter Kit – contains prepacked columns for each of the three chromatography steps (HiPrep™ 26/10 Sepharose 6 FF, HiTrap PlasmidSelect Xtra 5 ml, HiTrap SOURCE 30Q 5 ml). Facilitates process optimization, development and verification. Also produces mg amounts of high-quality supercoiled plasmid DNA (Fig 4).

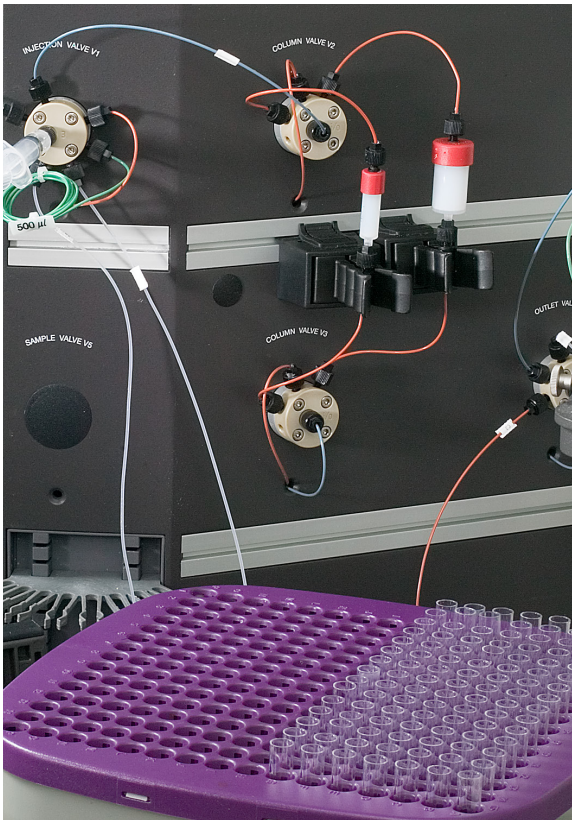

Fig 2. Plasmid DNA quantity and quality can be quickly assessed using PlasmidSelect Xtra Screening Kit and ÄKTAexplorer 10.

PlasmidSelect Xtra Screening Kit for rapid assessment of plasmid DNA quantity and quality

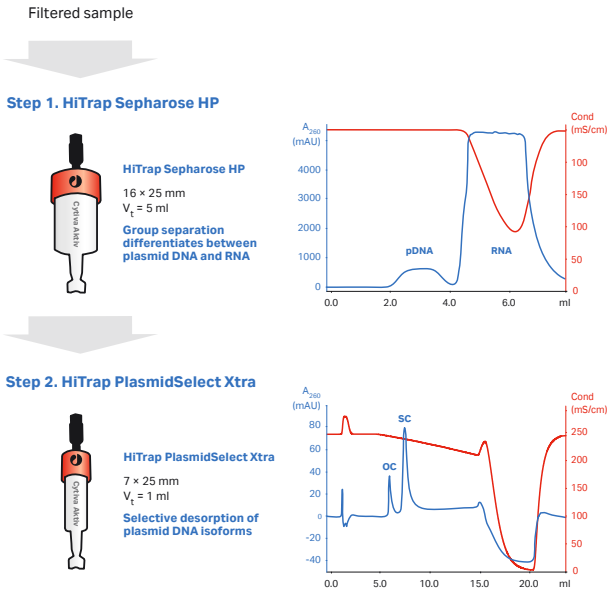

Fig 3. PlasmidSelect Xtra Screening Kit is used for fast and accurate determination of plasmid DNA quantity and quality.

OC = open circular; SC = supercoiled.

The PlasmidSelect Xtra Screening Kit is capable of determining the plasmid DNA content of any biological sample within 10 min and the ratio of supercoiled (sc) to open circular (oc) plasmid DNA within 30 min. It is particularly useful in situations that present practical difficulties in handling complex samples (e.g., fermentation, alkaline lysis, or anywhere in a plasmid DNA production process).

- Good performance in complex samples
- Reproducible and accurate results
- Quick and easy with an ÄKTA chromatography system

Purification with PlasmidSelect Xtra Starter Kit

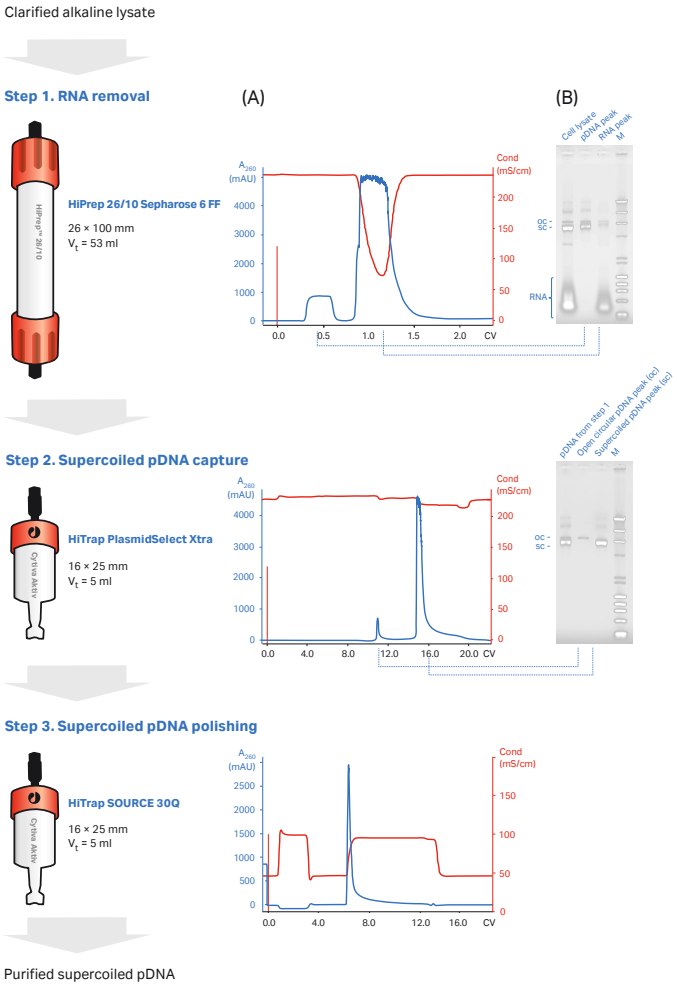

Fig 4. Purification of supercoiled plasmid DNA with PlasmidSelect Xtra Starter Kit. (A) Typical chromatograms of the three steps run on ÄKTAexplorer 10. (B) Ethidium bromide-stained 0.8% agarose electrophoresis gels of the key fractions eluted in Steps 1 and 2 indicate the purity of the fractions obtained after the different steps.

pDNA = plasmid DNA, oc = open circular, sc = supercoiled, covalently closed circular, M = molecular weight marker (DRIgest III code no. 27-4060-01).

The three prepacked columns in the starter kit are:

1. HiPrep 26/10 Sepharose 6 FF (53 ml)
2. HiTrap PlasmidSelect Xtra (5 ml)
3. HiTrap SOURCE 30Q (5 ml)

The PlasmidSelect Xtra Starter Kit contains sufficient media to purify up to 5 mg of high-quality, supercoiled plasmid DNA per cycle (Fig 4).

Kit instructions contain full experimental details for method design and optimization, as well as CIP and scale-up recommendations.

For convenience and efficiency, we recommend using a chromatography system with a gradient-forming pump and UV detector, preferably multiple wavelength, for example an ÄKTA system.

Scale-up of PlasmidSelect Xtra based process

All media in the PlasmidSelect Xtra Starter Kit are BioProcess media, specifically designed to meet the demands of industrial biotechnology. This means that the media are scalable from laboratory to production, are produced with validated manufacturing procedures, and can withstand standard cleaning-in-place (CIP) and sanitization-in-place procedures. In addition, BioProcess media are supported with regulatory support files and comprehensive documentation, as well as security of supply service.

The PlasmidSelect Xtra process allows the convenient transfer of preclinical plasmid DNA purification studies to large-scale, GMP-compliant production. It combines reproducibility and scalability with purity levels that meet gene therapy standards. Moreover, the process does not involve enzymes, precipitating agents, detergents, organic solvents or other components that require time-consuming removal or handling. All components can be re-used after CIP, and thanks to its robustness and scalability, the process can be easily automated at different scales.

Fig 5. Scale-up of PlasmidSelect Xtra process with pilot-scale columns connected to an ÄKTApilot™ system.

PlasmidSelect Xtra Screening Kit

The PlasmidSelect Xtra Screening Kit is designed for the rapid assessment of plasmid DNA quantity and quality. Plasmid DNA quantity is first determined by group separation on Sepharose High Performance and then plasmid DNA quality is verified by selective desorption of plasmid DNA isoforms from PlasmidSelect Xtra. Figure 3 shows this two-step procedure.

Determining plasmid DNA quantity

The method for determining the plasmid DNA content is based on group separation on HiTrap Sepharose HP (5 ml) and uses a standard curve showing the correlation between plasmid concentration and integrated peak area.

The method comprises equilibrating the HiTrap Sepharose HP column, loading and eluting the sample, collecting fractions, and integrating the peak area using UNICORN™ software. The plasmid DNA concentration in the sample can then be read from a standard curve prepared using known concentrations of plasmid DNA.

Figure 6 shows chromatograms obtained when plasmid samples of known concentration were run on HiTrap Sepharose HP and the standard curve generated from the obtained results. Each result was obtained in less than ten minutes and the figure clearly shows the reproducibility of the method. The very low variation confirms the robustness of this method.

Determining plasmid DNA quality

Determining plasmid quality is based on selective desorption of plasmid DNA isoforms from HiTrap PlasmidSelect Xtra following group separation on HiTrap Sepharose HP.

The method comprises adjusting conductivity of the partially purified plasmid DNA fraction from HiTrap Sepharose HP, equilibrating HiTrap PlasmidSelect Xtra, loading and desorbing the sample and finally calculating the relative amounts of supercoiled and open circular plasmid DNA by integrating the different peaks with UNICORN software. Note that a blank run is subtracted from the sample runs to obtain a straight baseline for peak integration (Fig 7). The direct analysis of the sample from HiTrap Sepharose HP is both convenient and accurate.

The application note *Fast determination of plasmid DNA quantity and quality in complex solutions* (code no. 28-4094-86) contains more detailed information. In addition, the PlasmidSelect Xtra Screening Kit can be used for fast preparation of high-quality plasmid DNA in analytical amounts.

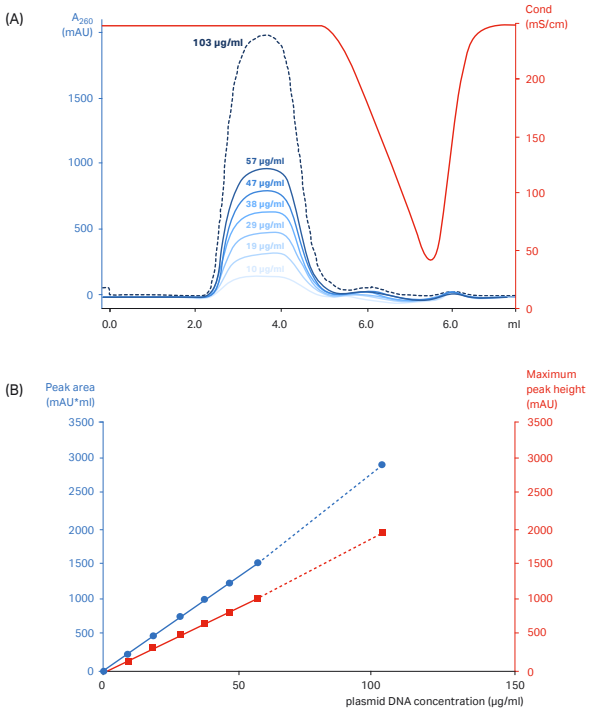

Fig 6. Fast determination of plasmid DNA content with the PlasmidSelect Xtra Screening Kit. (A) Sample runs of increasing concentrations of plasmid DNA. (B) Standard curves show the correlation between plasmid concentration and integrated peak area (circles) or maximum peak height (squares).

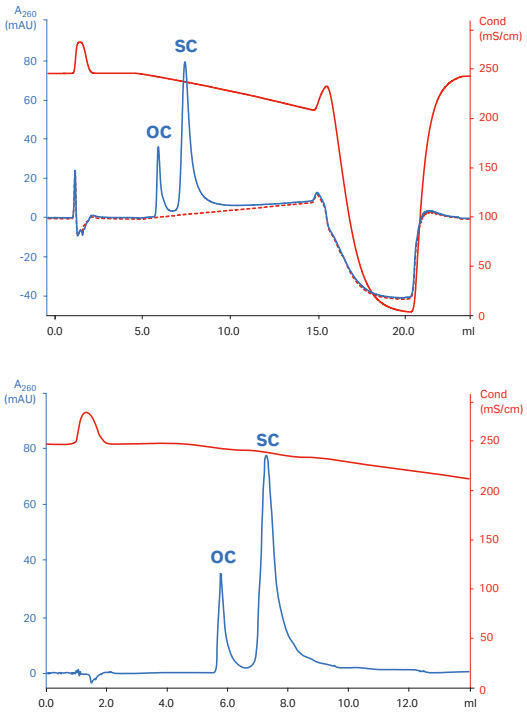

Fig 7. Evaluation of plasmid DNA quality with HiTrap PlasmidSelect Xtra. In the second chromatogram, the signal from a blank run has been subtracted from the plasmid DNA run.

Scale-up of the PlasmidSelect Xtra process

To purify larger amounts of plasmid DNA for preclinical and clinical studies, as well as routine production, scale up of the PlasmidSelect Xtra process is required. The recommended strategy here is to optimize conditions on the prepacked columns of the PlasmidSelect Xtra Starter Kit and then transfer to larger columns. Even though the change in bed dimensions might be significant, only minor modifications to the conditions should be needed to optimize the purification.

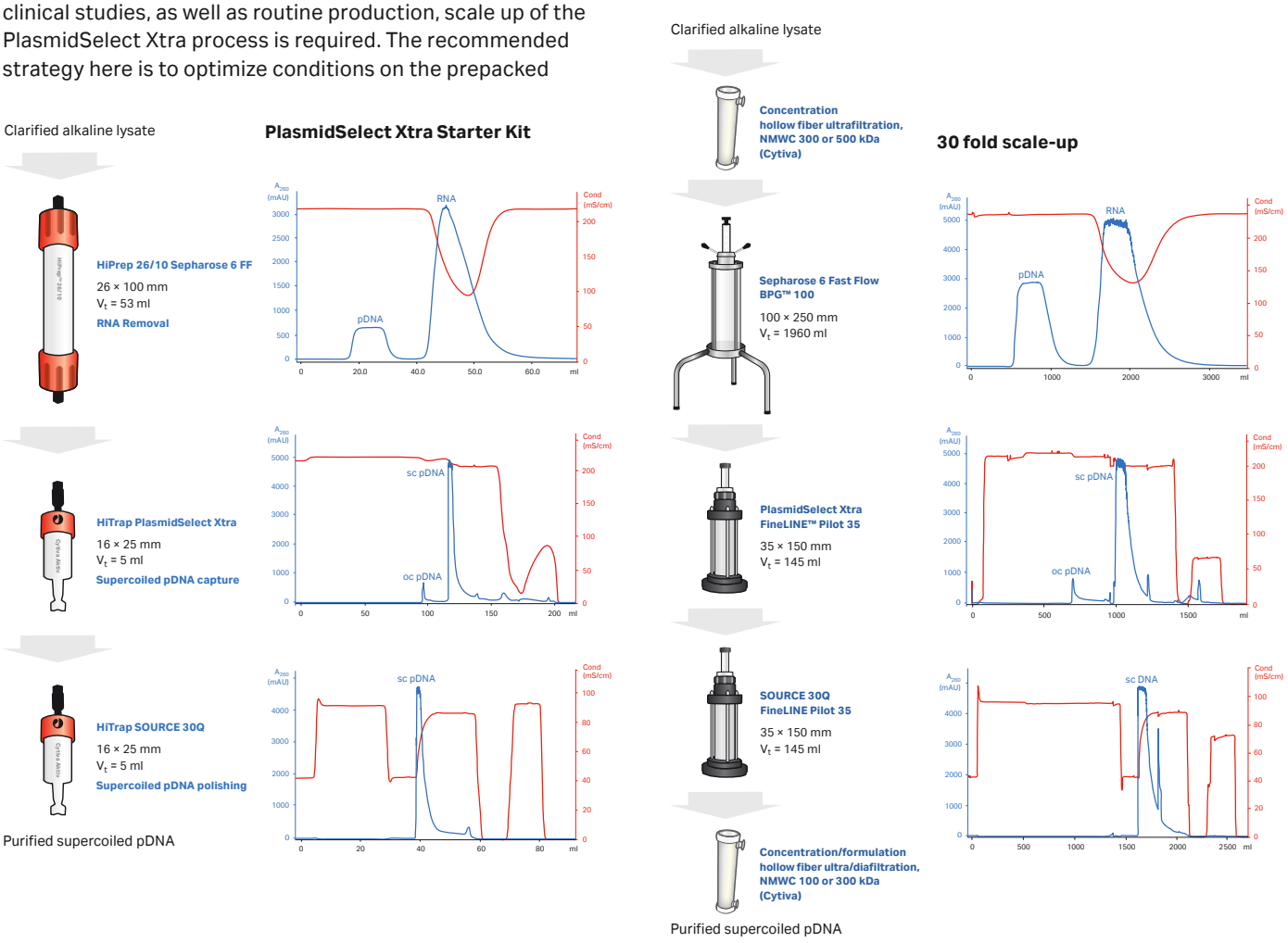

Fig 8. An approximately 30-fold scale up of the purification from the PlasmidSelect Xtra Starter Kit run on ÄKTAexplorer to bulk media packed in cGMP-compliant columns run on ÄKTApilot system.

Table 1. Analysis of the purified samples from the PlasmidSelect Xtra Starter Kit and from the pilot scale-run shown in Figure 8 (non GMP conditions)

Sample	pDNA conc (µg/ml)	% sc pDNA (CGE*)	EU Endotoxin/mg pDNA (LAL-test)	µg host cell protein/mg pDNA (BCA Protein Assay)	µg gDNA/mg† pDNA (TaqMan™ PCR)	µg RNA/mg pDNA (TaqMan PCR)
Purified samples from the PlasmidSelect Xtra Starter Kit run on ÄKTAexplorer 10 system						
Start material alkaline lysate	130	92	90 000	36 000	16.8	n.d.
Sepharose 6 Fast Flow group-separated material	129	94	10 000	< 39‡	0.54	0.0
PlasmidSelect Xtra supercoiled pDNA	606	98	220	< 8‡	0.35	0.0
SOURCE 30Q polished material	574	98	1.6	< 9‡	0.32	0.0
Analytical data for the purified sample from the pilot-scale run on ÄKTApilot system						
Final product (after 0.22 µm filtration)	4560	98	< 0.1§	< 1‡	0.02	0.01

* CGE = Capillary Gel Electrophoresis
† gDNA = genomic DNA
‡ Below detection limit (< 5 µg/ml)
§ Below detection limit (100× dilution: < 0.5 EU/ml)
The analyses were performed by PlasmidFactory, Bielefeld, Germany (*www.PlasmidFactory.com*)

Summary

By separating supercoiled plasmid DNA from open circular forms, PlasmidSelect Xtra chromatography medium forms the basis of a generic, scalable process for purifying high-quality supercoiled plasmid DNA with high throughput. The prepacked columns of the PlasmidSelect Xtra Starter Kit facilitate process optimization and verification. PlasmidSelect Xtra Screening Kit quickly and accurately determines plasmid DNA quantity and quality during process development.

The process is generic and robust and the DNA produced meets requirements for gene therapy and DNA vaccination. Cytiva supplies all key process needs, including chromatography media, dead-end and tangential flow filters, small and large prepacked columns, and fully scalable chromatography and filtration systems with 21 CFR part 11-compliant control software, as well as process development and regulatory support.

Media and prepacked column characteristics

Note that HiPrep and HiTrap columns cannot be opened or refilled.

Sepharose 6 Fast Flow, HiPrep 26/10 Sepharose 6 FF

Sepharose 6 Fast Flow performs first-step RNA removal and buffer exchange. The medium consists of 90 µm diameter highly cross-linked 6% agarose beads. It displays high chemical stability and is useful for group separation of large biomolecules such as RNA and plasmid DNA. In the PlasmidSelect Xtra Starter Kit, Sepharose 6 Fast Flow is prepacked as HiPrep 26/10 Sepharose 6 FF with a column volume of 53 ml. Table 2 describes key medium and column characteristics.

PlasmidSelect Xtra, HiTrap PlasmidSelect Xtra

The thiophilic aromatic adsorption medium PlasmidSelect Xtra captures and selectively desorbs supercoiled, covalently closed circular plasmid DNA. It consists of 34 µm diameter highly cross-linked agarose beads with an immobilized 2-mercaptopyridine ligand. In the starter and screening kits, it is prepacked as HiTrap PlasmidSelect Xtra with column volumes of 5 ml and 1 ml, respectively. Table 3 describes key medium and column characteristics.

SOURCE 30Q, HiTrap SOURCE 30Q

SOURCE 30Q medium consists of 30 µm diameter rigid, mono-sized polystyrene/divinyl benzene beads with quaternary ammonium groups. The ion exchange group is attached to the matrix via hydrophilic spacer arms following hydrophilization of the polymeric base matrix. SOURCE 30Q is used for reproducible, high-productivity separations at large-scale and is especially useful for final polishing.

In the PlasmidSelect Xtra Starter Kit, SOURCE 30Q is prepacked as HiTrap SOURCE 30Q 5 ml. Table 4 describes key medium and column characteristics.

HiTrap Sepharose HP

Sepharose High Performance medium consists of 34 µm diameter highly cross-linked 6% agarose beads. It displays high chemical stability and is useful for group separation of large biomolecules such as RNA and plasmid DNA. In the PlasmidSelect Xtra Screening Kit, Sepharose HP is prepacked as HiTrap Sepharose HP 5 ml. Table 5 describes key medium and column characteristics.

Table 2. Sepharose 6 Fast Flow and HiPrep 26/10 Sepharose 6 FF characteristics

Sepharose 6 Fast Flow	
Bead structure	Highly cross-linked agarose, 6%
Mean particle size	90 µm
Recommended flow rate for supercoiled plasmid DNA purification	30 to 60 cm/h
pH stability	
Working[1]	3 to 13
Cleaning[2]	2 to 14
Cleaning-in-place	≤ 1 M NaOH
Operating temperature	4°C to 40°C
Storage	4°C to 30°C in 20% ethanol

HiPrep 26/10 Sepharose 6 FF	
Bed volume	53 ml
Bed height	100 mm
Internal diameter	26 mm
Recommended flow rate[3]	2.7 to 27 ml/min (30 to 300 cm/h)
Recommended flow rate for supercoiled plasmid DNA purification	2.7 to 5.4 ml/min
Maximum flow rate[3]	40 ml/min (450 cm/h)

[1] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
[2] Refers to the pH interval for regeneration.
[3] Water at room temperature.

Table 3. PlasmidSelect Xtra and HiTrap PlasmidSelect Xtra characteristics

PlasmidSelect Xtra	
Bead structure	Highly cross-linked agarose, 6%
Mean particle size	34 µm
Ligand	2-mercaptopyridine
Ligand concentration	3.5 mg/ml
Dynamic binding capacity for supercoiled plasmid DNA (6125 bp)[1]	>2 mg/ml
Recommended flow rate in supercoiled plasmid DNA purification	≤ 120 cm/h
pH stability	
Working[2]	3 to 11
Cleaning[3]	2 to 13
Cleaning-in-place	0.5 M NaOH
Operating temperature	15°C to 30°C
Storage	4°C to 30°C in 20% ethanol

HITrap PlasmidSelect Xtra 1 ml (Screening Kit)	
Bed volume	1 ml
Bed height	25 mm
Internal diameter	7 mm
Recommended flow rate in supercoiled plasmid DNA purification	≤ 1 ml/min (150 cm/h)
Maximum flow rate	20 ml/min (600 cm/h)
Column hardware pressure limit[4]	0.5 MPa, 5 bar, 72 psi

HITrap PlasmidSelect Xtra 5 ml (Starter Kit)	
Bed volume	5 ml
Bed height	25 mm
Internal diameter	16 mm
Recommended flow rate in supercoiled plasmid DNA purification	≤ 4 ml/min (120 cm/h)
Maximum flow rate	20 ml/min (600 cm/h)
Column hardware pressure limit[4]	0.5 MPa, 5 bar, 72 psi

[1] Conditions for determining dynamic binding capacity.
Sample: Approx. 0.3 mg plasmid/ml in binding buffer (capacity at 10% breakthrough)
Column volume: 1 ml (Tricorn™ 5/50)
Flow rate: 0.2 ml/min
Binding buffer: 2.1 M $(NH_4)_2SO_4$, 10 mM EDTA, 100 mM Tris, pH 7.0
Elution buffer: 0.3 M NaCl, 1.7 M $(NH_4)_2SO_4$, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5
[2] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
[3] Refers to the pH interval for regeneration.
[4] Water at room temperature.

Table 4. SOURCE 30Q and HiTrap SOURCE 30Q characteristics

SOURCE 30Q	
Bead structure	Highly-rigid polystyrene/divinyl benzene
Mean particle size	30 µm
Functional group	Quaternary ammonium
Recommended flow rate in supercoiled plasmid DNA purification	≤ 120 cm/h
pH stability	
Working[1]	2 to 12
Cleaning[2]	1 to 14
Cleaning-in-place	≤ 1.0 M NaOH
Operating temperature Storage	4°C to 40°C 4°C to 30°C in 20% ethanol

HiTrap SOURCE 30Q 5 ml (Starter Kit)	
Bed volume	5 ml
Bed height	25 mm
Internal diameter	16 mm
Recommended flow rate in supercoiled plasmid DNA purification	≤ 4 ml/min (120 cm/h)
Maximum flow rate	20 ml/min (600 cm/h)
Column hardware pressure limit[3]	0.5 MPa, 5 bar, 72 psi

[1] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
[2] Refers to the pH interval for regeneration.
[3] Water at room temperature.

Table 5. HiTrap Sepharose HP characteristics

HiTrap Sepharose HP 5 ml (Screening Kit)	
Bead structure	Highly cross-linked agarose, 6%
Mean particle size	34 µm
Bed volume	5 ml
Bed height	25 mm
Internal diameter	16 mm
Recommended flow rate in sc pDNA process	< 4 ml/min
Maximum flow rate[1]	20 ml/min
Column hardware pressure limit	0.5 MPa, 5 bar, 72 psi
pH stability	
Working[2]	3 to 11
Cleaning[3]	2 to 13
Cleaning-in-place	0.5 M NaOH
Operating temperature	15°C to 30°C
Storage	4°C to 30°C in 20% ethanol

[1] Water at room temperature
[2] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
[3] Refers to the pH interval for regeneration.

Related equipment

Process development and scale up also requires hardware of different sizes such as larger chromatography columns (e.g., FineLINE™), and systems such as ÄKTAexplorer, ÄKTApilot and ÄKTAprocess™, plus filtration devices and equipment such as ÄKTAcrossflow™ and UniFlux™ systems, all of which are supplied by Cytiva.

Ordering information

Product	Pack size	Code number
PlasmidSelect Xtra Starter Kit	1 pack[1]	28-4052-68
PlasmidSelect Xtra Screening Kit	1 pack[2]	28-4052-69
PlasmidSelect Xtra	25 ml	28-4024-01
PlasmidSelect Xtra	200 ml	28-4024-02
PlasmidSelect Xtra	1 l	28-4024-03
PlasmidSelect Xtra	5 l	28-4024-04

[1] Contains one HiPrep 26/10 Sepharose 6 FF column, one HiTrap PlasmidSelect Xtra column and one HiTrap SOURCE 30Q column plus accessories. Does not include buffers.
[2] Contains five 5 ml HiTrap Sepharose HP columns and five 1 ml HiTrap PlasmidSelect Xtra columns plus accessories. Does not include buffers.

Related products	Pack size	Code number
Sepharose 6 Fast Flow	1 l	17-0159-01
Sepharose 6 Fast Flow	5 l	17-0159-04
Sepharose 6 Fast Flow	10 l	17-0159-05
SOURCE 30Q	50 ml	17-1275-01
SOURCE 30Q	200 ml	17-1275-02
SOURCE 30Q	1 l	17-1275-03
SOURCE 30Q	5 l	17-1275-04

Related literature	Code number
PlasmidSelect Xtra for downstream processing of supercoiled plasmid DNA, Application note	28-4094-85
Fast determination of plasmid DNA quantity and quality in complex solutions, Application note	28-4094-86
Sepharose 6 Fast Flow, Data file	18-1052-52
SOURCE 30Q, Data file	18-1107-12
Hollow fiber cartridges and systems for membrane separations, Selection handbook	18-1165-29

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