PlasmidSelect Xtra
PlasmidSelect Xtra Starter Kit

Instructions for Use

cytiva.com

28408202 AD

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Please read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or in vitro procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, please refer to the Safety Data Sheets.

1 Abstract

PlasmidSelect Xtra is the chromatography medium used in the selective capture step of a three-step chromatographic process to purify supercoiled, covalently closed circular plasmid DNA (sc pDNA).

This 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

PlasmidSelect Xtra Starter Kit contains these three BioProcess™ media in prepacked, ready to use columns in quantities sufficient to obtain up to 5 mg of pure supercoiled plasmid DNA.

The columns are:

HiPrep™ 26/10 Sepharose 6 FF, 53 ml

HiTrap™ PlasmidSelect Xtra, 5 ml

HiTrap SOURCE 30Q, 5 ml

Note that PlasmidSelect Xtra Starter Kit does not include buffers.

PlasmidSelect Xtra medium is also supplied in 25 and 200-ml laboratory packs as well as 1 and 5-l process-sized packs (see *Ordering Information*).

2 Introduction

PlasmidSelect Xtra medium and PlasmidSelect Xtra Starter Kit have been developed to purify supercoiled plasmid DNA. We recommend the three-step purification process using PlasmidSelect Xtra Starter Kit illustrated in Figure 1, Section 3, for obtaining pure supercoiled plasmid DNA. This process does not require the use of RNase enzyme. Figure 1 also shows typical results. The prepacked columns in the starter kit provide fast and easy separations in a convenient format. In addition, the process can be scaled up for cGMP purification of supercoiled plasmid DNA suitable for gene therapy.

Plasmid DNA content and quality can also be determined in a simple and easy way using PlasmidSelect Xtra Starter Kit (see Instructions 28408203).

3 Prepacked columns and media characteristics

HiPrep 26/10 Sepharose 6 FF columns and Sepharose 6 FF medium

HiPrep 26/10 Sepharose 6 FF column is prepacked with Sepharose 6 Fast Flow. This 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. Table 1 describes key column and medium characteristics.

Note: *HiPrep columns cannot be opened or refilled.*

Table 1. HiPrep 26/10 Sepharose 6 FF and Sepharose 6 Fast Flow characteristics

Medium	Sepharose 6 Fast Flow
Matrix	Highly cross-linked agarose, 6%
Average particle size	90 µm
Bed volume	53 ml
Bed height	100 mm
Internal diameter	26 mm
Column material	Polypropylene
Recommended flow velocity [1]	30 to 300 cm/h (2.7 to 27 ml/min)
Recommended flow velocity for sc pDNA purification	30 to 60 cm/h (2.1 to 5.3 ml/min)
Maximum flow velocity [1]	450 cm/h (40 ml/min)
Column hardware pressure limit	0.5 MPa, 5 bar, 73 psi
pH stability [2]	
Working range	3 to 13
Cleaning-in-place	2 to 14
Operating temperature	4°C to 40°C
Storage	20% ethanol at 4°C to 30°C

[1] H_2O at room temperature. For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow velocity is recommended.

[2] Working range: pH interval where the medium can be handled without significant change in function.

Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 2 lists the characteristics of HiTrap columns.

Note: *HiTrap columns cannot be opened or refilled.*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 2. Characteristics of HiTrap columns

Column volume (CV)	5 ml
Column dimensions	1.6 x 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16″ male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/16″	For sealing bottom of HiTrap column	2, 5 or 7

HiTrap PlasmidSelect Xtra and PlasmidSelect Xtra

HiTrap PlasmidSelect Xtra columns are prepacked with the thiophilic aromatic adsorption medium PlasmidSelect Xtra. This medium consists of 34-µm diameter highly cross-linked agarose beads with an immobilized 2-mercaptopyridine ligand. Its main application is purifying the supercoiled form of plasmid DNA. Table 3 describes key medium characteristics.

Table 3. HiTrap PlasmidSelect Xtra and PlasmidSelect Xtra characteristics

Matrix	Highly cross-linked agarose, 6%
Average particle size	34 µm
Ligand	2-mercaptopyridine, 3,5 mg/ml
Dynamic binding capacity for sc pDNA (6125 bp) [1]	> 2 mg/ml
Recommended flow velocity [2]	150 cm/h (5 ml/min)
Recommended flow velocity for sc pDNA purification	≤ 120 cm/h (4 ml/min)
Maximum flow velocity [2]	600 cm/h (20 ml/min)
pH stability [3]	
Working range	3 to 11
Cleaning-in-place	2 to 13
Cleaning-in-place	0.5 M NaOH
Operating temperature	15°C to 30°C
Storage	20% ethanol at 4°C to 30°C

[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 $(NH_4)_2SO4$, 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] H_2O at room temperature. For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow velocity is recommended.

[3] Working range: pH interval where the medium can be handled without significant change in function.

Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

HiTrap SOURCE 30Q and SOURCE 30Q

HiTrap SOURCE 30Q columns are prepacked with SOURCE 30Q medium that consists of 30-µm diameter rigid, mono-sized poly-styrene/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. Table 4 describes key medium characteristics.

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

Matrix	Highly rigid polystyrene/divinyl benzene
Average particle size	30 µm
Functional group	Quarternary ammonium
Recommended flow velocity [1]	150 cm/h (5 ml/min)
Recommended flow velocity for sc pDNA purification	≤ 120 cm/h (4 ml/min)
Maximum flow velocity [1]	600 cm/h (20 ml/min)
pH stability [2]	
Working range	2 to 12
Cleaning-in-place	1 to 14
Cleaning-in-place	≤ 1.0 M NaOH
Operating temperature	4°C to 40°C
Storage	20% ethanol at 4°C to 30°C

[1] H_2O at room temperature. For viscous buffers and samples the flow velocity must be optimized. Starting with a low flow velocity is recommended.

[2] Working range: pH interval where the medium can be handled without significant change in function.

Cleaning-in-place: pH interval where the medium can be subjected to cleaning-in-place without significant change in function.

4 Purification protocol

Flow scheme for PlasmidSelect Xtra Starter Kit

PlasmidSelect Xtra Starter Kit

Clarified alkaline lysate

HiPrep 26/10 Sepharose 6 FF

26 mm x 100 mm
Vt: 53 ml

RNA Removal

HiTrap PlasmidSelect Xtra

16 mm x 25 mm
Vt: 5 ml

Supercoiled pDNA capture

HiTrap SOURCE 30Q

16 mm x 25 mm
Vt: 5 ml

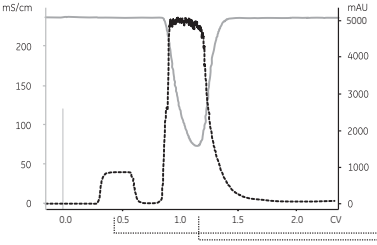
Supercoiled pDNA polishing

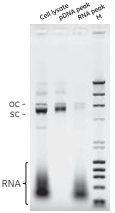
Purified supercoiled pDNA

A

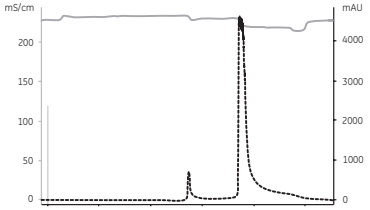
B

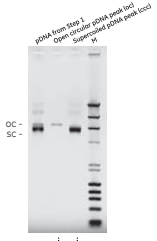
Step 1. HiPrep 26/10 Sepharose 6 FF





Step 2. HiTrap PlasmidSelect Xtra





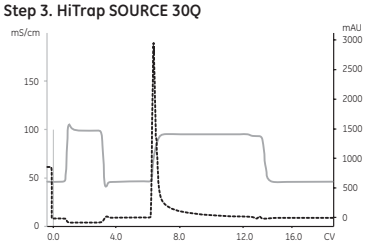


Fig 1. The process for purifying supercoiled plasmid DNA.

(A) Typical chromatograms of the three steps run on ÄKTAexplorer 10 (see *Flow scheme for PlasmidSelect Xtra Starter Kit, on page 9* for details).

(B) Ethidium bromide-stained 0.8% agarose electrophoresis gels of the key fractions eluted in Steps 1 and 2.

pDNA = plasmid DNA; oc = open circular; sc = supercoiled, covalently closed circular; M = molecular weight ladder (DRIgest III).

Equipment

For convenience and efficiency, we recommend using a chromato-graphy system with a gradient-forming pump and UV detection, for example ÄKTA™ systems. If you use ÄKTAexplorer, remove the flow restrictor to avoid high back-pressure when working with viscous samples such as clarified bacterial cell lysate. A temperature-compensated device to measure conductivity may prove useful. In addition, clarification by centrifugation or filtration may be required to prepare the bacterial alkaline cell lysate. You can assess the quality of the plasmid preparation after each step by running samples in 0.8% to 1% agarose gel electrophoresis and staining the gels with ethidium bromide[1].

1 Sambrook, J. and Russel, D. W., Molecular cloning. A laboratory manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (2001).

Buffers

You need the following buffers:

For Step A	
Buffer A:	2.1 M $(NH_4)_2SO_4$, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5
For Step B	
Buffer B:	2.0 M $(NH_4)_2SO_4$, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5
Buffer C:	0.3 M NaCl, 1.7 M $(NH_4)_2SO_4$, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5
For Step C	
Buffer D:	0.4 M NaCl, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5
Buffer E:	1.0 M NaCl, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5

Use high purity water and chemicals. Filter buffers through a 0.22-µm or 0.45-µm filter before use.

Sample preparation

The starting material for plasmid DNA (pDNA) is usually clarified bacterial cell lysate containing the desired plasmid1. Generally, fresh or frozen cell paste is suspended in 50 mM Tris-HCl buffer, pH 7.5, containing 10 mM EDTA to reduce DNAse activity and approximately 50 mM glucose. Lyse cells by adding 0.2 M NaOH, 1% SDS at room temperature. The suspension normally changes color and becomes viscous.

Note: *Perform this step quickly and use only gentle stirring to avoid shear stress breaking chromosomal DNA and damaging the plasmids.*

Flocculate cellular debris and SDS complexes by gently adding cold 3 M potassium acetate, pH 5 and incubating on ice. Clarify the plasmid DNA extract using filtration or centrifugation. This procedure normally gives an initial concentration of approximately 0.05 to 0.1 mg/ml plasmid DNA in an alkaline lysate.

To obtain up to 5 mg supercoiled plasmid DNA (sc pDNA) of high purity at the end of the three-step purification, the plasmid-containing extract may have to be concentrated using, for example, ultrafiltration. We suggest using hollow fiber ultrafiltration with a MWCO of 300, for example MidGee™ Cartridge UFP-300-C-MM12A from Cytiva. Typically, a plasmid DNA concentration from 0.5 to 1 mg/ml prior to RNA removal and buffer exchange on HiPrep 26/10 Sepharose 6 FF should minimize the number of cycles run on this column before capture of the supercoiled plasmid DNA on HiTrap PlasmidSelect Xtra.

Filter the sample through a filter (1 or 5 µm, for example) before applying it to the column.

You can readily scale up this process. For other column sizes and details of column packing, see Sections 4 and 5. Custom-packed columns are available on request (see *Ordering Information*).

Protocol

Step A: RNA removal and buffer exchange

Step	Action
1	Equilibrate HiPrep 26/10 Sepharose 6 FF with at least 110 ml (2 CV) of Buffer A at 2.7 to 5.3 ml/min (30 to 60 cm/h).
2	Load up to 16 ml (0.3 CV) of concentrated, clarified alkaline lysate at a flow rate of 2.7 ml/min (30 cm/h).
3	Collect the void volume containing the partially purified plasmid DNA preparation. Discard the RNA-containing second (large) fraction. ***Note:*** *RNA tends to elute with the yellowish colored band characteristic of the clarified cell lysate. Make sure that all material has eluted from the previous run before applying a new sample.*

Depending on the initial concentration of plasmid DNA, you can load several portions of the same sample on the column without extensive equilibration between consecutive loadings (see Fig 2). However, we recommend cleaning the column with water and re-equilibrate at least every fifth cycle or when the chromatogram becomes distorted.

For recommendations on media regeneration and cleaning-in-place (CIP), see *Maintenance*.

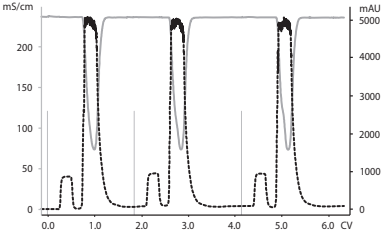


Fig 2. Three consecutive sample loadings on HiPrep 26/10 Sepharose 6 FF.

Step B: Capture and selective desorption of supercoiled plasmid DNA

Step	Action
1	Equilibrate HiTrap PlasmidSelect Xtra with at least 10 ml (2 CV) of Buffer B at 4 ml/min (120 cm/h).
2	Load the partially purified plasmid DNA sample from Step A at 4 ml/min (120 cm/h).
3	Wash with 20 ml (4 CV) of buffer B. Open circular forms of plasmid DNA are not retained and elute with the wash.
4	Elute supercoiled plasmid DNA with 25 ml (5 CV) of Buffer C (up to 3.3 ml/min [100 cm/h]).
5	Collect the peak of highly pure supercoiled plasmid DNA (Fig 1, Step 2).

For recommendations on media regeneration and cleaning-in-place (CIP), see *Maintenance*.

Step C: Final polishing

Step	Action
1	Equilibrate HiTrap SOURCE 30Q with at least 10 ml (2 CV) of Buffer D at 4 ml/min (120 cm/h).
2	Measure the volume of the supercoiled plasmid DNA eluate from Step B and dilute with two volumes of pure water or 100 mM Tris-HCl buffer, pH 7.5 to decrease the ionic strength of the sample.
3	Load the diluted sample at 4 ml/min (120 cm/h). ***Note:*** *The resulting conductivity may still be higher than in Buffer D.*
4	Wash with at least 10 ml (2 CV) of Buffer D and elute with at least 25 ml (5 CV) of Buffer E (up to 4 ml/min [120 cm/h]).
5	Collect the elution peak containing the purified supercoiled plasmid preparation (Fig 1, Step 3). ***Note:*** *The purified sample now contains more than 0.5 M NaCl.*

For recommendations on media regeneration and cleaning-in-place (CIP), see *Maintenance*.

5 Scaling up

Introduction

Optimize the purification using the small prepacked columns in the PlasmidSelect Xtra Starter Kit. After screening, optimizing, and verifying the method with PlasmidSelect Xtra Starter Kit, transfer the process to the bed heights required for further scale-up.

30-fold scale-up

Figure 3 shows a 30-fold scale-up from PlasmidSelect Xtra Starter Kit with increased bed heights but constant flow velocity and sample loading. Even though the change in bed height might be significant, only minor modifications to the conditions would be needed to further optimize the dynamic binding capacity/recovery for each specific plasmid. Scale-up details are stated opposite each image.

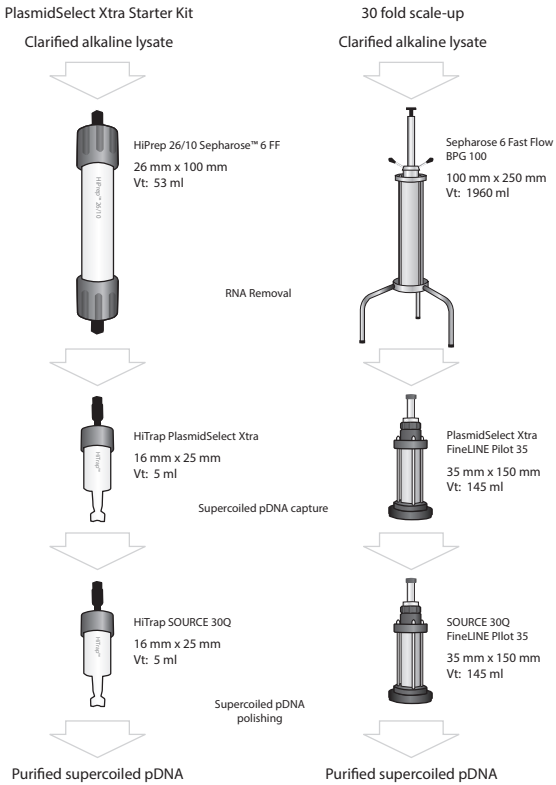


Fig 3. An approximately 30-fold scale-up from PlasmidSelect Xtra Starter Kit.

Sepharose 6 Fast Flow packed in BPG 100 column

Maintain		Increase	From	To
		Column bed height (mm)	100	250
		Column diameter (mm)	26	100
		Column volume (ml)	53	1960
Flow velocity (cm/h)	30-60	Volumetric flow rate (ml/min)	2.7-5.3	39-78
Equilibration (CV)	>2			
Sample loading (CV)	0.3	Sample volume (proportionally)	≤ 16 ml	≤ 590 ml

PlasmidSelect Xtra and SOURCE 30Q packed in FineLINE 35 column

Maintain		Increase	From	To
		Column bed height (mm)	25	150
		Column diameter (mm)	16	35
		Column volume (ml)	5	145
Flow velocity (cm/h)	90-120	Volumetric flow rate (ml/min)	3-4	14-19
Equilibration (CV)	>2	(ml)	10	290
Sample loading (CV)	Up to 2 mg/ml	Sample volume (proportionally)		

Further scaling up

It is possible to further scale-up of the purification process as outlined in the following image. Here both bed height and flow velocity are essentially kept constant, while bed diameter and volumetric flow rate are increased. Scale-up details are stated opposite each image.

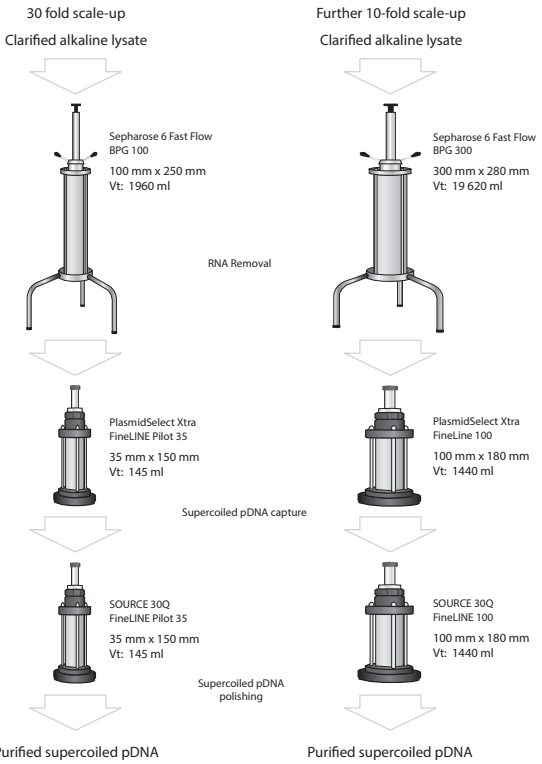


Fig 4. A further 10-fold scale-up from the purification process outlined in Fig 3.

Sepharose 6 Fast Flow packed in BPG 100 column

Maintain		Increase	From	To
		Column bed height (mm)	250	250
		Column diameter (mm)	100	100
		Column volume (ml)	1960	1960
Flow velocity (cm/h)	30-60	Volumetric flow rate (ml/min)	39-78	39-78
Equilibration (CV)	>2	(ml)	3900	40000
Sample loading (CV)	0.3	Sample volume (proportionally)	≤ 590 ml	≤ 5900 ml

PlasmidSelect Xtra and SOURCE 30Q packed in FineLINE 35 column

Maintain		Increase	From	To
		Column bed height (mm)	150	180
		Column diameter (mm)	35	100
		Column volume (ml)	144	1440
Flow velocity (cm/h)	90-120	Volumetric flow rate (ml/min)	14-19	130-157
Equilibration (CV)	>2		290	3000
Sample loading (CV)	Up to 2 mg/ml	Sample volume (proportionally)		

6 Packing columns for scaling up

Recommended pilot-scale columns

Medium	Empty column[1]
Sepharose 6 Fast Flow	XK 50/30[2], BPG 100
PlasmidSelect Xtra	XK 16/20, XK 26/20, FineLINE Pilot 35
SOURCE 30Q	XK 16/20[2], XK 26/20[3], FineLINE Pilot 35

[1] For inner diameter and maximum bed volumes and bed heights, see *Ordering Information*.

[2] Custom-packed columns are available on request, see *Ordering Information*.

[3] A special filter kit is needed, see *Ordering Information*.

The three media are supplied in 20% (v/v) ethanol. Prepare a slurry by decanting the 20% ethanol solution and replacing it with packing solution in a ratio of 50% to 70% settled medium to 50% to 30% packing solution. The packing solution should not contain agents that significantly increase viscosity. Distilled water or a low ionic strength buffer are suitable packing solutions for the media.

Packing XK columns

Step	Action
1	Equilibrate all materials to the temperature at which the chromatography will be performed.
2	De-gas the slurry to minimize the risk of air bubbles in the packed bed.

Step	Action
3	Eliminate air from the column dead spaces by flushing the end pieces with packing solution (or 20% ethanol). Make sure no air has been trapped under the column net. For SOURCE 30Q and an XK 16 column, place Filter HR 16 on top of the bottom plunger. For SOURCE 30Q and XK 26, place Filter 26 on the top of the bottom plunger.
4	Close the column outlet. Leave a few centimeters of packing remaining in the column.
5	To pack an XK 16 column: use Packing Connector XK 16 to connect an extra XK 16 column as a packing reservoir To pack an XK 26 column: use Packing Connector XK 26. To pack an XK 50 column: use RK 50 packing reservoir. Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the formation of air bubbles.
6	Immediately fill the remainder of the column with packing solution, mount the column top piece onto the column and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

Step	Action
7	Open the bottom outlet of the column and set the pump to run at the desired flow rate. **Packing Sepharose 6 Fast Flow in an XK 50/30 column** Let the gel (50% slurry in pure water) settle at a flow rate of 10 ml/min (30 cm/h) for 70 min, and then compress it at 30 ml/min (90 cm/h) for 10 min. Remove the Packing Connector and lower the adapter until it reaches the surface of the packed bed. Continue compressing at 60 ml/min (180 cm/h) for 10 min. **Packing PlasmidSelect Xtra in XK 16/20 or XK 26/20 columns** Pack at a constant flow rate of 5 ml/min (150 cm/h, XK 16/20) or 11.5 ml/min (130 cm/h, XK 26/20) in the first step, and 9 ml/min (270 cm/h, XK 16/20) or 21.2 ml/min (240 cm/h, XK 26/20) in the second step. Do not exceed 1.0 bar (0.1 MPa) in the first step and 3.5 bar (0.35 MPa) in the second step. **Packing SOURCE 30Q in XK 16/20 or XK 26/20 columns** Pack at 10 ml/min (300 cm/h) for the first 5 min (approx. 3 bar, 0.3 MPa). Then increase the flow rate until a maximum pressure of 5 bar (0.5 MPa) is achieved (20 to 30 ml/min [600 to 900 cm/h]). ***Note:*** *Do not exceed 70% of the packing flow rate in subsequent chromatographic procedures.*

Step	Action
8	Maintain the packing flow rate for 3 CV after a constant bed height is reached.
9	After packing is completed, mark the level of the packed bed on the column tube before stopping the pump. Then stop the pump and close the outlet. Disconnect the inlet tubing from the pump and remove the Packing Connector. For SOURCE 30Q, place Filter HR 16 on top of the packed bed. Then lower the adapter until it reaches the surface of the packed bed. Tighten the O-ring so that the adapter will slide when pushed. Finally, lower the adapter until it is approximately 3 mm below the mark on the column tube.

Packing FineLINE Pilot 35 column

For packing PlasmidSelect Xtra or SOURCE 30Q in a FineLINE Pilot 35 column, follow the packing procedures in the instructions supplied with the column.

Recommended process-scale columns

Medium	Empty column
Sepharose 6 Fast Flow	BPG, BioProcess LPLC and Chromaflow™
PlasmidSelect Xtra	FineLINE, BPG, BioProcess MPLC and Chromaflow
SOURCE 30Q	FineLINE and BioProcess MPLC

For process-scale columns, follow the packing procedures in the instructions supplied with the column.

7 Evaluating column packing

Introduction

To adhere to good laboratory practice (GLP) and good manufacturing practice (GMP), you should check the quality of the packing and monitor it during the working life of the column. Test column efficiency directly after packing, at regular intervals afterwards, and when separation performance is seen to deteriorate. The methods we recommend for expressing the efficiency of a packed column are height equivalent to a theoretical plate, HETP, and asymmetry factor, A_s. These values are easily determined by applying a sample such as 1.0 M NaCl in water with 0.5 NaCl in water as eluent.

Note: *The calculated plate number varies according to test conditions and you should therefore use it only as a reference value. It is also important that you keep conditions and equipment constant so that results are comparable. Changes in solute, solvent, eluent, sample volume, flow rate, liquid pathway, temperature, etc., will influence the results.*

For optimal results, make sure that the sample volume does not exceed 2.5% of the CV, and keep the flow velocity between 15 and 30 cm/h.

If an acceptance limit is defined in relation to column performance, you can use the column plate number as part of the acceptance criteria for column use.

Method for measuring HETP and asymmetry factor (A_s)

To avoid diluting the sample, apply it as close to the column inlet as possible.

Conditions	
Sample volume	1% of CV
Sample concentration	1 M NaCl
Eluent	0.5 M NaCl in water, or dilute buffer
Flow velocity	30 cm/h
Detection NaCl, buffer	Conductivity

Calculating HETP and A_S

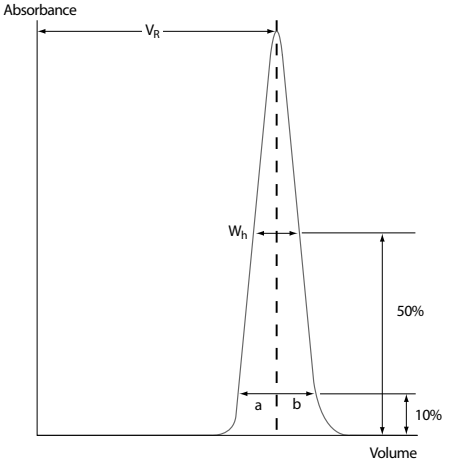


Fig 5. A typical test chromatogram showing HETP and A_S value calculations.

Calculating HETP

$$HETP = L/N$$

and

$$N = 5.54 (V_e/W_h)^2$$

where

L = Bed height (cm)

N = Number of theoretical plates

V_e = Peak elution distance

W_h = Peak width at half peak height

V_e and W_h are in the same units.

To facilitate comparing column performance, the concept of reduced plate height is often used.

Reduced plate height (h) is calculated as:

$$h = HETP/d$$

where

d is the mean diameter of the beads. As a guide, a value of h between 2 and 4 indicates good performance.

The peak should be symmetrical, and the asymmetry factor as close to one as possible. A change in the shape of the peak is usually the first indication of bed deterioration due to use.

Calculating A_S

$$A_S = b/a$$

where

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height

A_S = 0.8 to 1.5 (guide)

8 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Figure below. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 1, on page 5, Table 3, on page 7 and Table 4, on page 8) may damage the column.*

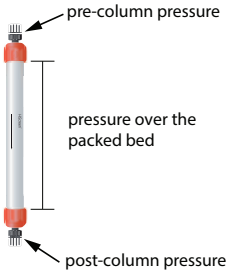


Fig 6. Pre-column and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see *Table 1, on page 5* and *Table 2, on page 6*).

The maximum pressure the packed bed can withstand depends on medium characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

Step	Action
1	Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2	Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.

Step	Action
3	Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see *Table 1, on page 5* and *Table 2, on page 6*). Replace the pressure limit in the software with the calculated value. The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Note: *Repeat the procedure each time the parameters are changed.*

9 Maintenance

Regeneration

For best performance, wash bound substances from the column after each chromatographic cycle (see Table 5).

Table 5. Recommended conditions for column regeneration

Column	Wash	Volume (ml)	Re-equilibration with starting buffer (ml)
HiPrep 26/10 Sepharose 6 FF	Water	110 (2 CV)	110-160 (2-3 CV)
HiTrap PlasmidSelect Xtra	Water	10 (2 CV)	10-15 (2-3 CV)
HiTrap Source 30Q	2 M NaCl	10 (2 CV)	10-15 (2-3 CV)

To prevent the slow build up of contaminants on the column over time, more rigorous cleaning protocols may be needed on a regular basis.

Cleaning-in-Place (CIP)

CIP is the removal of very tightly bound, precipitated or denatured substances generated in previous purification cycles. If such contaminants accumulate on the column, they may affect its chromatographic properties.

Use the following CIP protocols as a guide when formulating a cleaning protocol specific for the raw material used. The frequency of CIP will depend on the amount of raw material applied to the column. Nevertheless, we recommend CIP after every cycle when purifying plasmids from clarified alkaline cell lysate.

HiPrep 26/10 Sepharose 6 FF

Step	Action
1	Wash with at least 110 to 160 ml (2 to 3 CV) of water at a flow rate of 2.7 to 5.3 ml/min (30 to 60 cm/h).
2	Wash with 110 to 160 ml (2 to 3 CV) of 0.5 M NaOH at a low flow rate of 0.4 to 1.3 ml/min (5 to 15 cm/h).
3	Incubate the column for at least 15 min.
4	Wash with at least 110 to 160 ml (2 to 3 CV) of water at a flow rate of 2.7 to 5.3 ml/min (30 to 60 cm/h).
5	Re-equilibrate the column with 110 to 160 ml (2 to 3 CV) of Buffer A.

HiTrap PlasmidSelect Xtra

Step	Action
1	Wash with at least 10 to 15 ml (2 to 3 CV) of water at a flow rate of 4 ml/min (120 cm/h).
2	Wash with 10 to 15 ml (2 to 3 CV) of 0.5 M NaOH at a low flow rate of 1.3 ml/min (40 cm/h).
3	Incubate the column for at least 15 min.
4	Wash with at least 10 to 15 ml (2 to 3 CV) of water at a flow rate of 4 ml/min (120 cm/h).
5	Re-equilibrate the column with 10 to 15 ml (2 to 3 CV) of Buffer B.

HiTrap SOURCE 30Q

Step	Action
1	Wash with at least 10 to 15 ml (2 to 3 CV) of water at a flow rate of 4 ml/min (120 cm/h).
2	Wash with 10 to 15 ml (2 to 3 CV) of 0.5 M NaOH at a low flow rate of 1.3 ml/min (40 cm/h).
3	Wash with 10 ml (2 CV) of 2.0 M NaCl at a low flow rate of 1.3 ml/min (40 cm/h).
4	Incubate the column for at least 15 min.
5	Wash with at least 10 to 15 ml (2 to 3 CV) of water at a flow rate of 4 ml/min (120 cm/h).

Step	Action
6	Re-equilibrate the column with 10 to 15 ml (2 to 3 CV) of Buffer D.

Sanitization

Sanitization is the reduction of microbial contamination in the column and related equipment to an acceptable minimum. You need to design a specific sanitization protocol for each process according to the type of contaminants present and local regulatory demands. We recommend washing the column with 0.5 M NaOH with a contact time of 30 to 60 min.

Storage

Store media and columns at 4°C to 30°C in 20% ethanol.

10 Ordering Information

Product	Quantity	Code No.
PlasmidSelect Xtra Starter Kit	1 pack[1]	28405268
PlasmidSelect Xtra	25 ml	28402401
	200 ml	28402402
	1 l	28402403
	5 l	28402404

[1] Contains one HiPrep 26/10 Sepharose 6 FF column, one HiTrap PlasmidSelect Xtra column, one HiTrap Source 30Q column and accessories.

Accessories	Quantity	Code No.
1/16" male/luer female	2	18111251
(For connection of syringe to top of HiTrap column)		
Tubing connector flangeless/M6 female	2	18100368
(For connection of tubing to bottom of HiTrap column)		
Tubing connector flangeless/M6 male	2	18101798
(For connection of tubing to top of HiTrap column)		
Union 1/16" female/M6 male	6	18111257
(For connection to original FPLC System through bottom of HiTrap column)		
Union M6 female /1/16" male	5	18385801
(For connection to original FPLC System through top of HiTrap column)		
Union luerlock female/M6 female	2	18102712
HiTrap/HiPrep, 1/16" male connector for ÄKTA	8	28401081
Stop plug female, 1/16"	5	11000464
(For sealing bottom of HiTrap column)		
Fingertight stop plug, 1/16"	5	11000355

Related media products	Quantity	Code No.
Sepharose 6 Fast Flow	1 l	17015901
	10 l	17015905
SOURCE 30Q	50 ml	17127501
	200 ml	17127502
	1 l	17127503
	5 l	17127504
PlasmidSelect Xtra Screening Kit	1 pack[1]	28405269

[1] Contains five 5-ml HiTrap Sepharose HP columns, five 1-ml HiTrap PlasmidSelect Xtra columns, and accessories.

Custom-packed columns	Code No.
Sepharose 6 Fast Flow XK 50/30	90100368
SOURCE 30Q XK 16/20	90100380

Empty pilot-scale columns and accessories	Code No.
XK 16/20 (16 mm i.d.)	18877301
Max. 30 ml bed volume or 15 cm bed height	
XK 26/20 (26 mm i.d.)	18100072
Max. 65 ml bed volume or 12.5 cm bed height	
XK 50/30 (50 mm i.d.)	18875101
Max. 550 ml bed volume or 28.5 cm bed height	
FineLINE Pilot 35	18110202
Max. 144 ml bed volume or 15 cm bed height	
BPG 100/500 (100 mm i.d.)	18110301
Max. 2 l bed volume or 26 cm bed height	
Packing Connector XK16	18115344
Packing Connector XK 26	18115345
XK 50 Reservoir	18879001
XK 50 Fast Flow Kit	18100070
Filter Kit HR 16	18358501
Filter Kit 26	18110128
Online Filter (50 and 100 ml/min system)	18111244
Online Filter Kit (20, 50, and 100 ml/min systems)	18102711

DNA molecular weight markers	Code No.
DRIgest III (restriction enzyme digest of DNA, 25 µg)	27406001

Related literature	Code No.
Data File, Sepharose 6 Fast Flow	18105252
Data File, SOURCE 30Q	18110712
Data File, BPG 100, 140, 200, 300	18111523

Related literature	Code No.
Data File, BPG 450	18106059
Data File, Chromaflow	18113892
Data File, FineLINE Pilot 35	18110495
Data File, FineLINE 100/100L, 200/200L	18113000
Data File, BioProcess LPLC columns	18116776
Data File, BioProcess MPLC columns	18116775
Data File, Hollow fiber Start AXM and Start AXH cross flow cartridges for the ÄKTAcrossflow automated system	18116809
Selection Handbook, Hollow fiber cartridges and systems for membrane separations.	18116529

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