

PlasmidSelect Xtra Screening Kit

Instructions for Use

PlasmidSelect Xtra is a chromatography medium with a selectivity that allows supercoiled (sc) covalently closed circular forms of plasmid DNA (pDNA) to be separated from open circular (oc) forms. PlasmidSelect Xtra Screening Kit is for fast screening and quantification of plasmid DNA in complex biological samples.

The workflow comprises:

1. Determining plasmid DNA quantity by group separation on Sepharose™ High Performance.
2. Determining plasmid DNA quality by selective desorption of plasmid DNA isoforms from PlasmidSelect Xtra.

PlasmidSelect Xtra Screening Kit contains these two media in prepacked, ready to use HiTrap™ columns.

The columns are:

- HiTrap Sepharose HP, 5 ml
- HiTrap PlasmidSelect Xtra, 1 ml

Each kit contains 5 x 5 ml HiTrap Sepharose HP and 5 x 1 ml HiTrap PlasmidSelect Xtra columns plus accessories. Note that PlasmidSelect Xtra Screening Kit does not include buffers. PlasmidSelect Xtra medium is also supplied in 25-ml and 200-ml laboratory packs as well as 1-l and 5-l process-sized packs (see [Chapter 6 Ordering information, on page 18](#)).

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1 Introduction

PlasmidSelect Xtra Screening Kit uses a two-step procedure to conveniently determine plasmid DNA content and quality in a simple and fast way in situations where other methods have difficulty in handling complex samples, e.g. fermentation, alkaline lysis, or anywhere in a pDNA production process.

You can determine the plasmid DNA content of any biological sample within 10 min and the ratio sc/oc plasmid DNA within 30 min. In addition, you can use the kit for fast preparation of high-quality plasmid DNA in analytical amounts.

The kit works according to the same principle as PlasmidSelect Xtra medium and PlasmidSelect Xtra Starter Kit, which have both been developed for purifying supercoiled (sc) plasmid DNA (see *Instruction 28408202*).

For PlasmidSelect Xtra Screening Kit, we recommend the two-step flow scheme illustrated in [Fig. 3, on page 9](#), which also shows examples of typical results obtained when analyzing an alkaline lysate.

In addition, this procedure also produces small quantities of pure plasmid DNA that can be collected for further investigations.

For developing a plasmid DNA purification process, we recommend PlasmidSelect Xtra Starter Kit and bulk media (see [Chapter 6 Ordering information, on page 18](#)).

2 Product description

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 1, on page 4](#) lists the characteristics of HiTrap columns.



Fig 1. HiTrap, 1 ml column.



Fig 2. HiTrap, 5 ml column

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

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

Table 1. Characteristics of HiTrap columns

Column volume (CV)	1 ml	5 ml
Column dimensions	0.7 × 2.5 cm	1.6 × 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	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 Sepharose HP and Sepharose High Performance

HiTrap Sepharose HP columns are prepacked with Sepharose High Performance. This medium consists of 34- μ m 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, on page 4](#) describes key column and medium characteristics.

Table 2. HiTrap 26/10 Sepharose HP and Sepharose High Performance characteristics

Medium	Sepharose High Performance
Matrix	Highly cross-linked agarose, 6%
Average particle size	34 μ m
Recommended flow rate ¹	5 ml/min
Recommended flow rate for sc pDNA processing	< 4 ml/min
Maximum flow rate ¹	20 ml/min
pH stability ²	
Working range	3 to 11
Cleaning-in-place	2 to 13

Operating temperature	15°C to 30°C
Storage	20% ethanol at 4°C to 30°C

¹ H₂O 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 PlasmidSelect Xtra, 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 purification of the supercoiled form of plasmid DNA. [Table 2, on page 5](#) describes key column and 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 rate²	1 ml/min
Recommended flow rate for sc pDNA purification	≤ 1 ml/min
Maximum flow rate²	600 cm/h (20 ml/min)
pH stability³	
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
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- 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 (NH₄)₂SO₄, 10 mM EDTA, 100 mM Tris, pH 7.0
 Elution buffer: 0.3 M NaCl, 1.7 M (NH₄)₂SO₄, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5
- ² H₂O 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.

3 Purification protocol

Flow scheme



HiTrap Sepharose HP

16 mm x 25 mm

Vt: 5 ml

Group separations

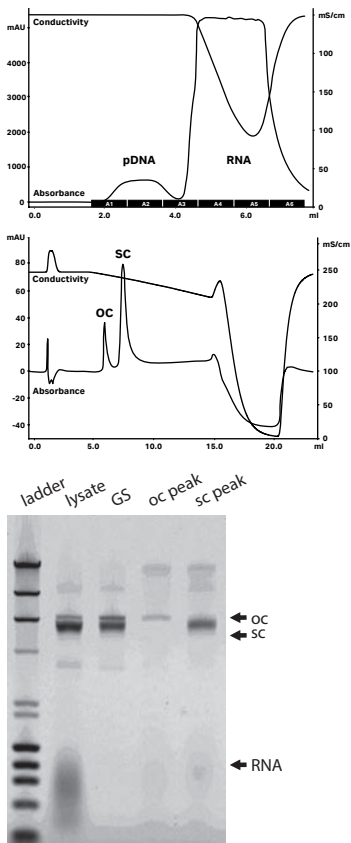


HiTrap PlasmidSelect Xtra

7 mm x 25 mm

Vt: 1 ml

Selective desorption of
plasmid DNA isoforms



Electrophoresis

Ethidium bromide-stained 0.8% agarose electrophoresis gel of key fractions eluted in Steps 1 and 2.

oc = open circular, sc = supercoiled, covalently closed circular. RNA = ribonucleic acid. Ladder = molecular weight ladder (Amersham DRIgest III).

Fig 3. Process for purifying plasmid DNA using PlasmidSelect Xtra Screening Kit.

Equipment

For convenience and efficiency, we recommend using a chromatography 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.¹

Buffers

You need the following buffers:

For Step A

Buffer A: 2.1 M (NH₄)₂SO₄, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5

For Step B

Buffer B: 2.0 M (NH₄)₂SO₄, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5

Buffer C: 0.3 M NaCl, 1.7 M (NH₄)₂SO₄, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5

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

If the plasmid DNA is in a soluble form, no sample preparation is needed.

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

Note: When working with organic solvents, precipitate and redissolve the plasmid DNA in aqueous buffer¹. When working with cells, alkaline cell lysis¹ is required to solubilize the plasmid DNA.

In general, suspend fresh or frozen cell paste 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 0.02 to 0.1-mg/ml plasmid DNA in an alkaline lysate. If necessary, filter the sample through a filter (e.g., 1 or 5 µm) before applying it to the column.

Protocol

Step A: Plasmid DNA content

Determining plasmid content is based on group separation on HiTrap Sepharose HP.

Step	Action
------	--------

- | | |
|---|--|
| 1 | Equilibrate HiTrap Sepharose HP with at least 10 ml (2 column volumes [CV]) of Buffer A at 2 ml/min. |
|---|--|

Step	Action
------	--------

- | | |
|---|---|
| 2 | Load 1.5 ml (0.3 CV) of sample at a flow rate of 2 ml/min. |
| 3 | Collect the void volume in three 1-ml fractions starting at 1.5 ml after injection. The void volume contains the partially purified plasmid DNA (see Fig. 1, on page 4 , Step 1). |

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.

- | | |
|---|--|
| 4 | Integrate the peak as described in the UNICORN™ user documentation and read the concentration from a standard curve. |
|---|--|

Note:

Develop a standard curve by running samples of known concentrations (e.g. 0, 10, 20, 30, 40, 50, 60, and 100 µg/ml) as described above. See [Fig. 2, on page 4](#).

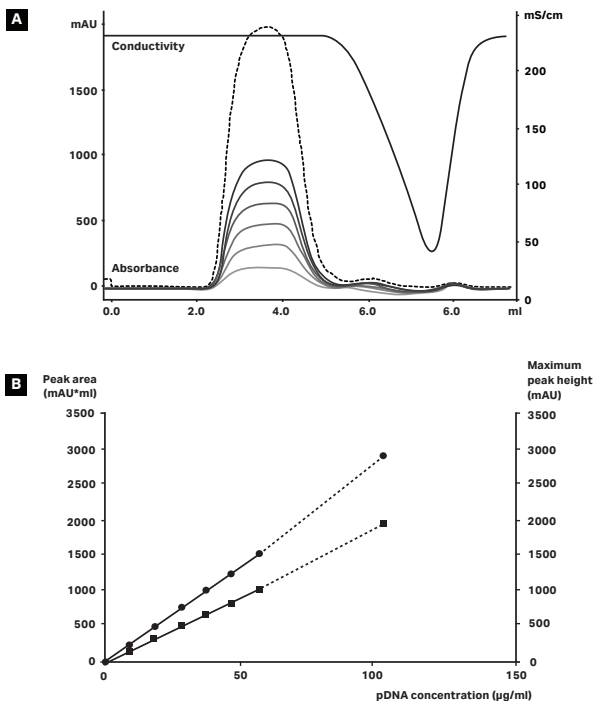


Fig 4. Chromatograms and corresponding standard curves for pDNA.

(A) Standard sample runs of known concentrations.

(B) Standard curves show the correlation between plasmid concentration and integrated peak area (dots) and maximum peak height (squares).

Step B: Plasmid DNA quality

Determining plasmid quality is based on selective desorption of plasmid DNA isoforms from HiTrap PlasmidSelect Xtra.

Step	Action
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- | | |
|---|--|
| 1 | Prepare the sample by diluting the pDNA fraction from the previous step (Fig. 1, on page 4 , Step 1) with an equal volume of Buffer E (500 µl plasmid DNA sample + 500 µl Buffer E is recommended). |
| 2 | Equilibrate HiTrap PlasmidSelect Xtra with at least 5 ml (5 CV) of Buffer B at 0.4 ml/min. |
| 3 | Load 0.5 ml of the partially purified plasmid DNA sample from Step 1 at 0.4 ml/min and wash with 2 ml (2 CV) of Buffer B. Collect fractions for further characterization if needed. |
| 4 | Elute with an 11-ml gradient of 0–55% Buffer C (11 CV) followed by 5 ml of water. |
| 5 | Calculate the ratio of supercoiled vs. open circular plasmid DNA by integrating the different peaks as described in the UNICORN user documentation. |

Note:

Start by running a blank on the column. Use this run to subtract the blank curve from sample runs to obtain a straight baseline for peak integration. See also [Fig. 5, on page 15](#).

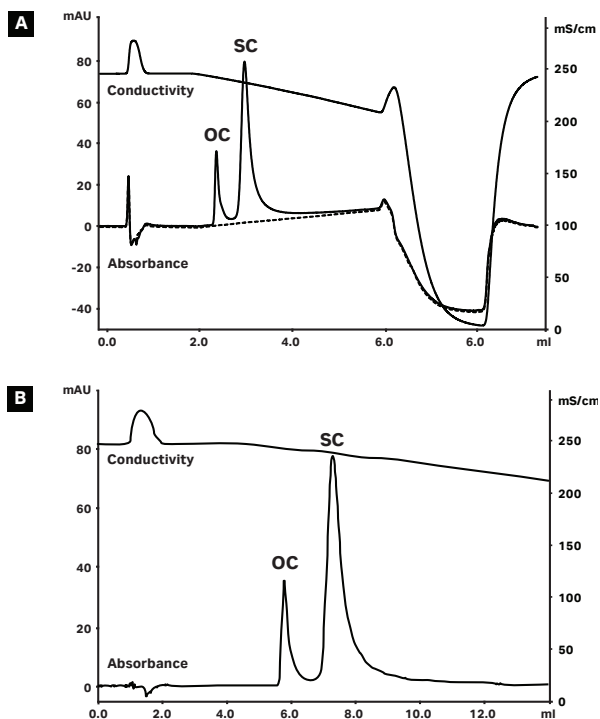


Fig 5. Evaluation of plasmid DNA quality with PlasmidSelect Xtra.

- (A)** Original sample (solid line) run with a blank (dashed line).
(B) Signal from the blank run is subtracted from the plasmid DNA run and the curve zoomed.

4 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 2, on page 5](#) and [Table 3, on page 6](#)) may damage the column.

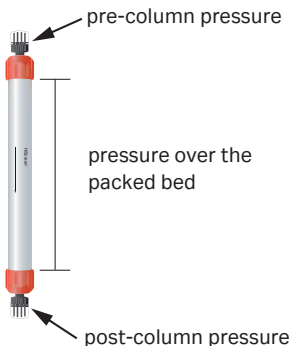


Fig 6. Pre-column and post-column measurements.

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 4](#)).

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, ÄKTAFLC 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 <i>total system pressure</i> , 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 4](#)). 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.

5 Storage

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

6 Ordering information

Product	Quantity	Code No.
PlasmidSelect Xtra Screening Kit	1 pack ¹	28405268

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

Accessories	Quantity	Code No.
1/16" male/luer female	2	18111251

(For connection of syringe to top of HiTrap column)

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

Related media products	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
Sepharose 6 Fast Flow	1 l	17015901
	5 l	17015904
	10 l	17015905

Related media products	Quantity	Code No.
SOURCE™ 30Q	50 ml	17127501
	200 ml	17127502
	1 l	17127503
	5 l	17127504

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

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28408203 AC V:7 09/2020