PlasmidSelect Xtra

Product Information

Cat#No#	PI-427C

Product Overview

PlasmidSelect Xtra is a thiophilic aromatic adsorption chromatography resin with a selectivity that allows supercoiled covalently closed circular forms of plasmid DNA to be separated from open circular forms.
PlasmidSelect Xtra allows for generic purification of supercoiled plasmid DNA.
PlasmidSelect Xtra allows for consistent purification results when scaling up from research scale to cGMP production.
Available in convenient prepacked formats for research, process development and quantitative and qualitative analysis of supercoiled plasmid DNA.
The hydrophilic nature of the base matrix ensures low levels of non-specific binding.
This resin is a BioProcess resin supported for industrial applications.

Description

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.

Characteristic

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.

Maximum operating pressure

< 120 cm/h, XK 16/20 column.

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CREATIVE BIOMART®
Chromatography

PlasmidSelect Xtra

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. 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.
Matrix
cross-linked agarose
Particle Size
24 µm-44 µm
Average particle size
~34 µm
Ligand
2-mercaptopyridine
Ligand density
Approx. 3.5 mg 2-mercaptopyridine/ml medium.
Dynamic binding capacity
≤ 120 cm/h
Recommended flow rate
30 to 300 cm/h (2.7 to 27 ml/min)
Recommended column height
100 mm
Chemical stability

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Stable in commnly used aqueous buffers
pH working range
3–11
CIP stability
2–13
Temperature stability
15°C to 30°C
Storage
4 to 30°C, 20% Ethanol
Evaluation of Packing
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, As . 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.
Regeneration
We recommend washing the column with 0.5 M NaOH with a contact time of 30 to 60 min.
Cleaning-in-place
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.
Scaling up
The three media are supplied in 20% (v/v) ethanol. Prepare a slurry by decanting the 20% ethanol solution

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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.

Pack size
25 mL
BioProcess resin
Yes
Maximum flow velocity
450 cm/h (40 ml/min)
Dimensions
26 mm
Column volume
53 ml
Column hardware pressure limit
0.5 MPa, 5 bar, 72 psi

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