# **Capto** PlasmidSelect

PLASMID PURIFICATION RESINS

Capto™ PlasmidSelect 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. The resin is designed for purification of supercoiled DNA to high quality for gene therapy and DNA vaccine applications. Compared with its predecessor PlasmidSelect Xtra resin, Capto PlasmidSelect is based on a more rigid base matrix, delivering excellent pressure-flow properties to plasmid production. The possibility to run at higher flow rates and bed heights increases flexibility in process design and allows for an increased productivity. Capto PlasmidSelect is available in bulk as well as in formats suitable for process development (Fig 1).

**Key benefits of Capto PlasmidSelect include:**

- High-flow purification of supercoiled DNA from research scale to cGMP production
- Flexible process design due to a large operational window of flow velocities and bed heights
- Higher throughput for improved productivity and process economy
- Hydrophilic properties of base matrix prevent non-specific binding
- BioProcess™ resin supported for industrial applications

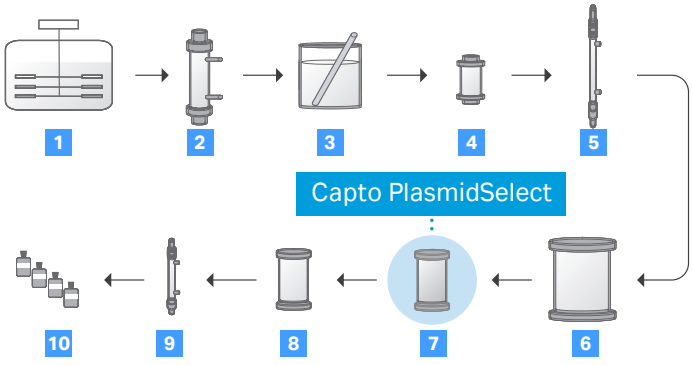
## Product overview

Purified plasmid DNA is required in increasing quantities to meet emerging requirements for gene therapy and DNA vaccine applications. 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.

Capto PlasmidSelect chromatography resin forms the basis of a generic process for purifying supercoiled covalently closed circular plasmid DNA suitable for bulk as well as for clinical-grade applications. The process provides high capacity, delivers high yields, and can be scaled up to fulfill requirements for cost-efficient industrial manufacture of plasmid DNA in regulated environments.


**Fig 1.** Capto PlasmidSelect plasmid purification resin.

The same principle can also be used to rapidly analyze the quantity and quality of plasmid DNA in complex solutions. Figure 2 shows a schematic illustration of the plasmid purification process, including the Capto PlasmidSelect capture step.




1 Fermentation
2 Cell harvesting
3 Lysis
4 Clarification
5 Concentration
6 RNA removal
7 Supercoiled plasmid DNA
8 Polishing
9 Concentration
10 Formulation

**Fig 2.** Process for production of high-quality supercoiled plasmid DNA from fermentation to formulation.

## Bead size optimized for high-flow processes

Capto PlasmidSelect is based on the well-established Capto high-flow agarose base matrix, which demonstrates excellent pressure-flow properties. The rigid matrix allows for high flow velocities in modern downstream purification processes (Fig 3). The hydrophilic nature of the resin ensures low levels of non-specific binding.

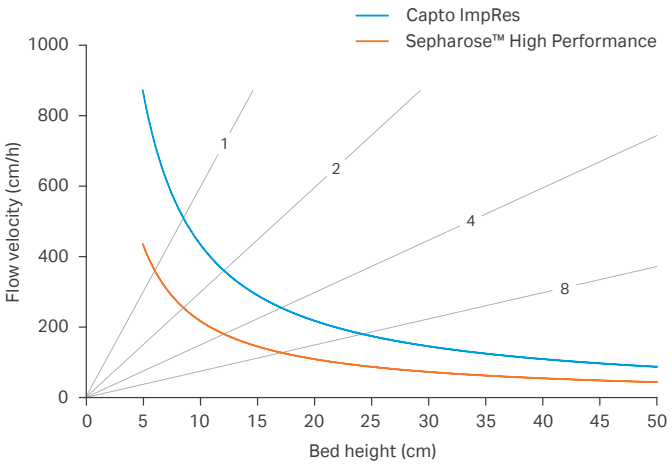



**Fig 3.** The window of operation (area under the curve) of Capto ImpRes (Capto PlasmidSelect) and Sepharose High Performance (PlasmidSelect Xtra) base matrices. Data corresponds to a 1 m diameter column at 20°C and viscosity equivalent to water. Gray contours show the residence time in the column in minutes.

## Facilitated process scale-up

Capto PlasmidSelect is a BioProcess resin specifically designed to meet the demands of industrial applications. The resin is available prepacked in HiTrap™ and HiScreen™ columns suitable for process development and optimization as well as for laboratory-scale preparative purifications. Capto PlasmidSelect is also available in bulk for scale-up to manufacturing scale (Fig 4).

Capto PlasmidSelect is produced with robust manufacturing procedures, and can withstand standard cleaning-in-place (CIP) and sanitization-in-place methods. As a BioProcess resin, Capto PlasmidSelect is supported with regulatory support files and comprehensive documentation, as well as security of supply services.

Capto PlasmidSelect provides reproducibility and scalability with purity levels that meet gene therapy standards. For scale-up of the plasmid purification process, the recommended strategy is to optimize conditions using the prepacked Capto PlasmidSelect columns, and then transfer the process to larger-scale columns. Even though the change in bed dimensions might be significant, only minor modifications to the conditions will be needed to optimize the purification.

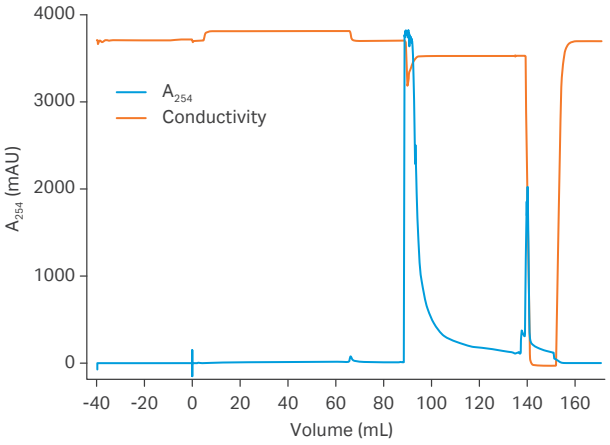
**Fig 4.** Capto PlasmidSelect plasmid purification processes can be scaled from laboratory to production scale.

## Enhanced productivity

A more rigid agarose resin allows for increased flow rates as well as the possibility to pack higher column beds, both enabling improved productivity. Increasing flow rate over the whole chromatographic purification process, (i.e., during column packing, conditioning, load, wash, elution, regeneration, cleaning-in-place, and reconditioning) can reduce total processing time substantially. Using higher column beds with the same diameter, more plasmid can be purified during the same cycle, which increases throughput. For example, going from a 15 cm bed height (PlasmidSelect Xtra) to a 20 cm bed hight (Capto PlasmidSelect) results in a 33% increase in resin volume and consequently 33% more product can be processed per cycle if the capacity of the resins is the same. Altogether, the use of a more rigid chromatography resin, such as Capto PlasmidSelect, results in a significant improvement in downstream process productivity.

Chromatograms from runs using Capto PlasmidSelect and PlasmidSelect Xtra are shown in Figure 5. Although the resins provide comparable performance, Capto PlasmidSelect allows for higher sample load at higher flow velocity.

(A) *Columns:* HiScreen Capto PlasmidSelect
*Sample:* Plasmid-containing feedstream
*Equilibration:* 100 mM Tris, 2 M ammonium sulfate, 10 mM EDTA, pH 7.5
*Sample load:* 3.0 mg/mL resin (61.27 mL) at 200 cm/h
*Wash:* 5 column volumes (CV) of 100 mM Tris, 2 M ammonium sulfate, 10 mM EDTA, pH 7.5 at 100 cm/h
*Elution:* 10 CV of 100 mM Tris, 1.7 M ammonium sulfate, 10 mM EDTA, pH 7.5 + 0.3 M NaCl at 80 cm/h
*Strip:* 3 CV 100 mM Tris, 10 mM EDTA at 100 cm/h
*System:* ÄKTA™ avant 150




(B) *Columns:* HiScreen PlasmidSelect Xtra
*Sample:* Plasmid-containing feedstream
*Equilibration:* 100 mM Tris, 2 M ammonium sulfate, 10 mM EDTA, pH 7.5
*Sample load:* 2.0 mg/mL resin (40.85 mL) at 100 cm/h
*Wash:* 5 CV of 100 mM Tris, 2 M ammonium sulfate, 10 mM EDTA, pH 7.5 at 100 cm/h
*Elution:* 10 CV of 100 mM Tris, 1.7 M ammonium sulfate, 10 mM EDTA, pH 7.5 + 0.3 M NaCl at 80 cm/h
*Strip:* 3 CV 100 mM Tris, 10 mM EDTA at 100 cm/h
*System:* ÄKTA avant 150

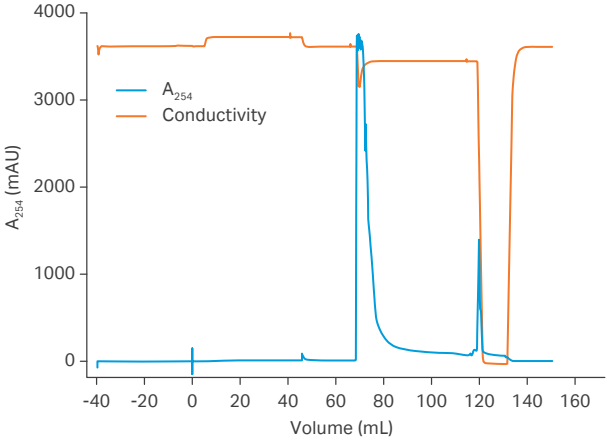



**Fig 5.** Chromatograms from plasmid purification using (A) Capto PlasmidSelect and (B) PlasmidSelect Xtra. Chromatograms are based on data from Cobra Biologics.

# Product specifications

The main characteristics of the resin and columns are summarized in Tables 2 and 3, respectively.

**Table 1.** Resin characteristics.

| | **Capto PlasmidSelect** |
|---|---|
| Matrix | Highly cross-linked agarose, spherical |
| Ligand | 2-mercaptopyridine |
| Ligand concentration | 27–50 µmol/mL |
| Particle size, $d_{50v}$* | 36–44 µm |
| Dynamic binding capacity† | ≥ 3.0 mg/mL |
| Recommended flow velocity in supercoiled plasmid DNA purification | 220 cm/h‡ |
| pH stability, operational§ | 3 to 13 |
| pH stability, CIP¶ | 2 to 14 |
| Chemical stability | Stable in up to 1 M NaOH, 40% Isopropanol, up to 1 M acetic acid, and up to 70% ethanol. |
| Delivery conditions | 20% ethanol |
| Storage | 20% ethanol at temperatures between 4°C and 30°C. |

* Median particle size of the cumulative volume distribution.
† Conditions for determining dynamic binding capacity (DBC) were as listed in Fig 5A.
‡ Packed in a 1 m diameter column at 20 cm bed height, using buffers with the same viscosity as water at 20°C at < 3 bar (0.3 MPa).
§ pH range where resin can be operated without significant change in function.
¶ pH range where resin can be subjected to cleaning-in-place (CIP) without significant change in function.

**Table 2.** Column characteristics.

| | **HiScreen** | **HiTrap, 1mL** | **HiTrap, 5 mL** |
|---|---|---|---|
| Column volume | 4.7 mL | 1 mL | 5 mL |
| Column dimensions | 0.77 × 10 cm | 0.7 × 2.5 cm | 1.6 × 2.5 cm |
| Column hardware pressure limit | 8 bar (0.8 MPa) | 5 bar (0.5 MPa) | 5 bar (0.5 MPa) |
| Recommended operating flow rate* | 0.8-2.3 mL/min | 1 mL/min | 5 mL/min |
| Maximum operating flow rate* | < 2.3 mL/min | < 4 mL/min | < 20 mL/min |

* At room temperature in buffers with the same viscosity as water at 20°C.

### Cleaning and sanitization

CIP is a procedure that removes tightly bound impurities and contaminants, such as lipids, precipitates, or denatured proteins, generated from the sample and that can remain in the column after regeneration. Regular CIP prevents the build-up of these contaminants and helps maintain the capacity, flow properties, and general performance of the resin. A specific CIP protocol should be designed for each process according to the type of contaminants that are present in the feed stream. General recommendation for CIP and sanitization of Capto PlasmidSelect is to use 1.0 M NaOH. Use of a water-diluted organic solvent, such as ethanol or isopropanol, can be efficient in breaking strong hydrophobic interactions during CIP.

## Acknowledgment

We thank Cobra Biologics for kindly sharing data and for valuable discussions.

## Ordering information

| Product | Size | Product code |
|---|---|---|
| Capto PlasmidSelect | 25 mL | 17549901 |
| | 100 mL | 17549902 |
| | 1 L | 17549903 |
| | 5 L | 17549904 |
| HiTrap Capto PlasmidSelect | 5 × 1 mL | 17549911 |
| | 1 × 5 mL | 17549912 |
| HiScreen Capto PlasmidSelect | 4.7 mL | 29201790 |
| **Related products** | | |
| Data file: HiScreen prepacked columns | | 28930581 |

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