# Purification of oligonucleotides using Capto™ Q ImpRes

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## Abstract

Our goal was to develop a robust, scalable and fully water-based purification for the purification of a majority of synthetically produced, and fully deprotected oligonucleotides utilizing low pressure (< 3 bar) bioprocessing equipment. In this study Capto™ Q ImpRes is compared to SOURCE™ 30Q, with data for purity, yield and pool volume. The purification process is verified with a 21-mer DNA sequence 5′ ACG TTG CAG ACT CCT AAT GGT 3′ and a 3′ N-Acetylgalactosamine (GalNac) modified version of the same sequence.

## Introduction

The synthesis of oligonucleotides has been boosted in recent years due to an increased need for new and powerful drugs, e.g. antisense DNA, aptamers, siRNA and miRNA, acting as modulators of protein expression or function. Even though the monomer coupling efficiency throughout the synthesis is high, contaminating species such as short-mers are produced. These impurities show high resemblance to the target molecule which makes the purification challenging, requiring high resolution.

Purification of oligonucleotides have often involved RPC, HIC or anion exchangers on small beads e.g. SOURCE™ 30Q resin. Neither of the mentioned techniques are easy scalable into semi large or large scale. In this work the purification of oligonucleotides using Capto™ Q ImpRes is presented and compared to SOURCE™ 30Q, which has been an industry standard for many years. Capto™ ImpRes is a modern, agarose based and scalable resin suitable for packing in AxiChrom™ columns ranging up to > 1 m in diameter (Fig 1). The quaternary ammonium ligand (Q) gives a strong anion functionality.

In this study Capto™ ImpRes with a multimodal ligand, adhere, was also evaluated. The adhere ligand act simultaneously with anion exchanger and hydrophobic interaction (HIC) functionality. High pH was employed to resolve secondary structure formation. For RNA and other oligonucleotides sensitive to high pH it is recommended to instead employ neutral pH conditions and resolve secondary structures with chaotropic buffers.

## Materials and methods

### Resin screening

Three resins from Cytiva were screened—Capto™ Q ImpRes, SOURCE™ 30Q, and Capto™ adhere ImpRes. The particle size is 40 µm for the Capto™ ImpRes resins and 30 µm for SOURCE™ 30Q. Table 1 lists the running conditions. The screening was performed using ÄKTA™ avant 25.

**Table 1.** Running conditions

| | |
|---|---|
| *Column* | HiScreen™, bed height = 10 cm, CV = 4.7 mL |
| *Equilibration* | 3 CV of 10 mM NaOH |
| *Load* | 1 mL (8.2 mg/mL) D21 oligo nucleotide |
| *Wash* | 8 CV of 10 mM NaOH |
| *Elution* | 0% to 100% of 10 mM NaOH in 2 M NaCl, in 40 CV |
| *Flow rate* | 0.78 mL/min |

### Head-to-head comparison

From the screening, Capto™ Q ImpRes and SOURCE™ 30Q were picked for a head-to-head comparison. Table 2 lists the running conditions.

**Table 2.** Running conditions, head-to-head comparison

| | |
|---|---|
| *Column* | HiScreen™, bed height = 10 cm, CV = 4.7 mL |
| *Equilibration* | 3 CV of 10 mM NaOH |
| *Load* | 10 mL (1.7 mg/mL) D21 oligo nucleotide |
| *Wash* | 4 CV of 10 mM NaOH |
| *Elution* | 0% to 50% of 10 mM NaOH in 2 M NaCl, pH 11, in 20 CV |
| *Flow rate* | 1.16 mL/min (150 cm/h, 4 min residence time) |

### Scale-up and verification run

To verify the robustness of the method and resin, two additional runs were preformed using Capto™ Q ImpRes; a scale-up run and one with GalNac-modified oligo. For the GalNac-run, the conditions were identical as for the head-to-head run besides the load was 6 mL (61.5 OD/mL or 2.6 mg/mL) GalNac-modified D21 oligo.

**Table 3.** Running conditions, scale-up

| | |
|---|---|
| *Column* | HiScale™ 26, bed height = 11 cm, CV = 62 mL |
| *Equilibration* | 3 CV of 10 mM NaOH |
| *Load* | 300 mL (1.7 mg/mL) D21 oligo nucleotide |
| *Wash* | 4 CV of 10 mM NaOH |
| *Elution* | 0% to 50% of 10 mM NaOH in 2 M NaCl, in 20 CV |
| *Flow rate* | 10 mL/min (113 cm/h, 6.1 min residence time) |

## Abstract

Capto™ Q ImpRes and SOURCE™ 30Q have similar fingerprints according to Figure 3, much different from Capto™ adhere ImpRes. When operating them side-by-side, both perform very good in terms of purity, yield, and pool volume (Table 4). For most of the runs Capto™ Q ImpRes showed slightly better pressure/flow properties which is verified in Figure 1.

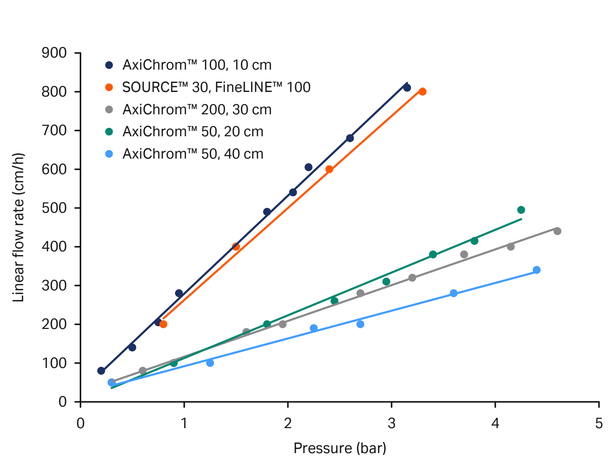



**Fig 1.** Pressure-flow curves for AxiChrom™ pilot scale columns packed to different bed heights with Capto™ ImpRes resin. Measurements were made with water at 20°C. The contribution of hardware pressure is excluded. Different chromatography resin batches were used to cover a large operational space.

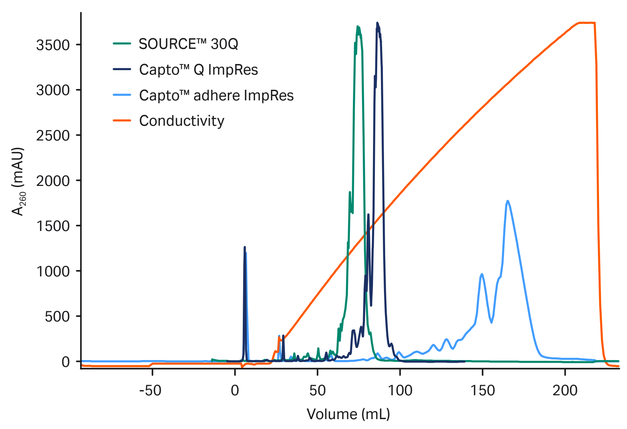
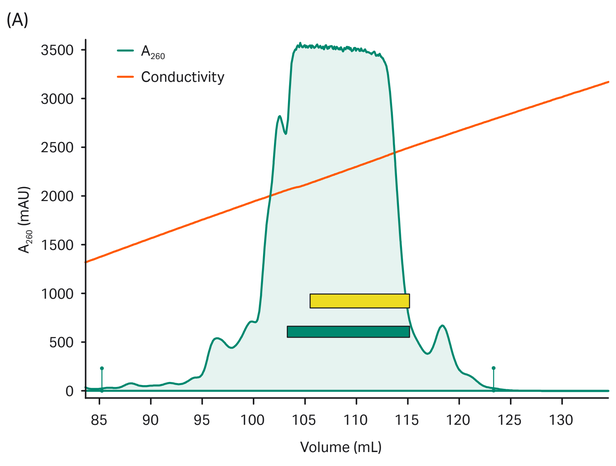



**Fig 3.** Cromatographic finger-print of Capto™ Q ImpRes, Capto™ adhere ImpRes and SOURCE™ 30Q when purifying a D21 oligonucleotide in a NaCl-gradient, 0–2 M in 40 CV.



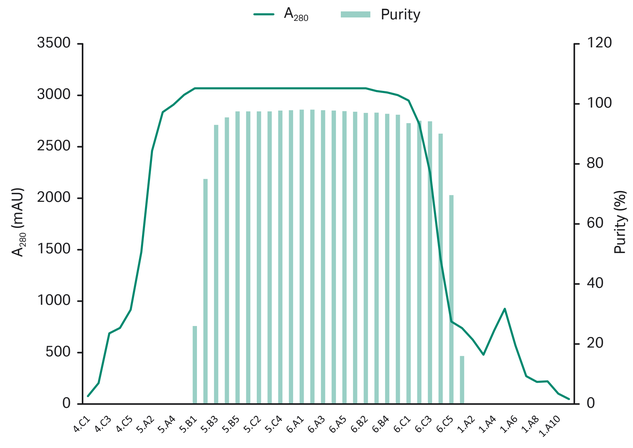




**Fig 4.** Distribution of full-length oligo during the scale-up run.

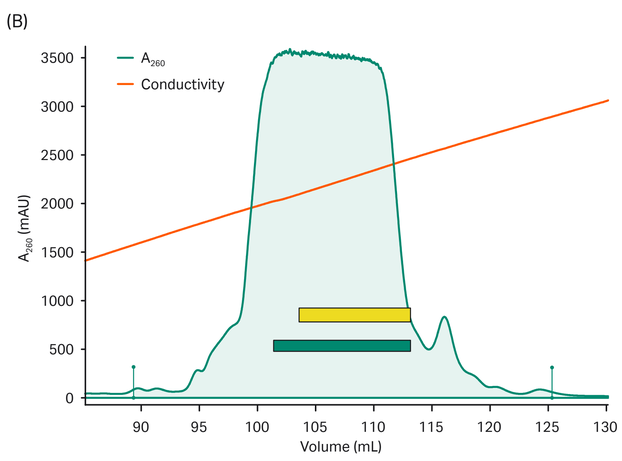



**Fig 2.** Comparison of Capto™ Q ImpRes and SOURCE™ 30Q for the separation and pooling of D21 oligonucleotide in a NaCl-gradient, 0–1 M in 20 CV.

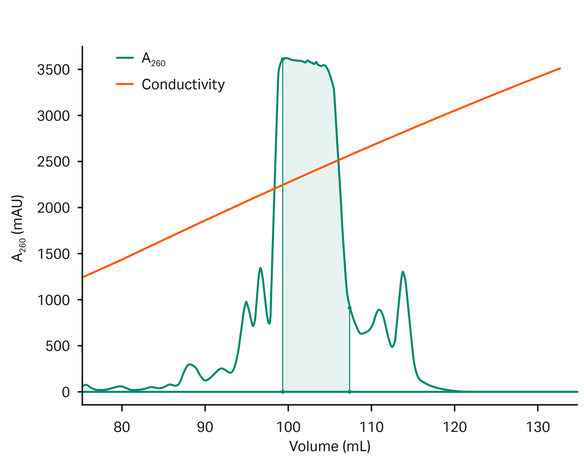



**Fig 5.** Chromatogram showing the purification of GalNac-modified oligo in a NaCl-gradient, 0–1 M in 20 CV.

**Table 4.** Summary of responses for various runs

| Sample ID | Load (mg/mL) | Yield (%) | Purity (%) | Pool volume (CV) | Figure |
|---|---|---|---|---|---|
| Crude sample | | | 86.6 | | |
| Capto™ Q ImpRes, yellow bar | 3.7 | 82 | 94.6 | 2.1 | Figure 2A |
| Capto™ Q ImpRes, green bar | 3.7 | 99 | 90.9 | 2.5 | Figure 2A |
| SOURCE™ 30Q, yellow bar | 3.7 | 76 | 96.2 | 2.1 | Figure 2B |
| SOURCE™ 30Q, green bar | 3.7 | 96 | 92.8 | 2.5 | Figure 2B |
| Capto™ Q ImpRes, scale-up | 8.2 | 80 | 95.2 | 3.2 | Figure 4 |
| Capto™ Q ImpRes, GalNac-modified | 2.6 | 98 | 81.5 | 1.7 | Figure 5 |

## Conclusion

Capto™ Q ImpRes has, for oligonucleotide purification, been shown to have similar properties as SOURCE™ 30Q with regards to resolution and yield. The favorable scalability characteristics associated with Capto™ Q ImpRes makes it an attractive choice for large scale downstream processing of oligonucleotides utilizing standard bioprocessing equipment (e.g. AxiChrom™ columns and prepacked ReadyToProcess™ columns) at low pressure (< 3 bar) conditions. The multimodal resin Capto™ adhere ImpRes gives a different chromatographic fingerprint than anion exchange resins. This gives opportunities for alternative and complementing purification strategies for specific oligonucleotide sequences. Attractive yield and resolution with Capto™ Q ImpRes was confirmed also with a GalNac modified DNA oligonucleotide.

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