

## One-Step Purification of Research-Grade Single-Stranded mRNA

TN0013

### Technical Note

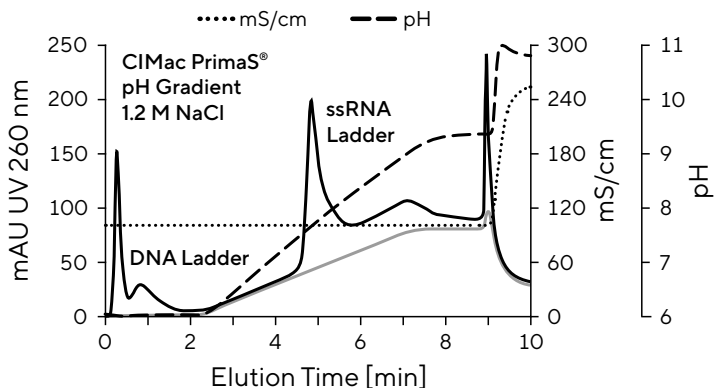


### Introduction

CIMac PrimaS<sup>®</sup> is a new member of BIA's family of high performance monoliths for analysis and purification of mRNA. Its positive charge gives it some anion exchange behavior but hydrogen bonding makes its selectivity is entirely distinct from traditional anion exchangers. QA and DEAE anion exchangers need to be heated into the range of 50–70°C for large mRNA to elute. PrimaS<sup>®</sup> elutes mRNA in a pH gradient, well separated from DNA and dsRNA [1]. New experimental data show that NaCl shifts the elution profile to lower pH values.

Experiments were conducted on a 100  $\mu$ L CIMac PrimaS<sup>®</sup> monolith at a flow rate of 10 CV/min (600 CV/hr). Columns were equilibrated with 20 mM MES, 20 mM tris, 20 mM BTP, 20 mM glycine, 1.2 M NaCl, pH 6.0. They were eluted with a linear gradient to 20 mM MES, 20 mM tris, 20 mM BTP, 20 mM glycine, 1.2 M NaCl, pH 10.0, then cleaned with 1 M NaOH + 2 M NaCl. Figure 1 shows results with a sample mixture containing an ssRNA ladder (200 b–6 kb) and a DNA ladder (80 bp–10 kbp). Figure 2 shows separation of an in vitro transcription (IVT) mixture. Both figures show double-stranded species being unretained or only weakly retained and eliminated early in the gradient. Single-stranded mRNA elutes at about pH 8, well separated from DNA and dsRNA. Proteins do not bind PrimaS<sup>®</sup> in 1.2 M NaCl.

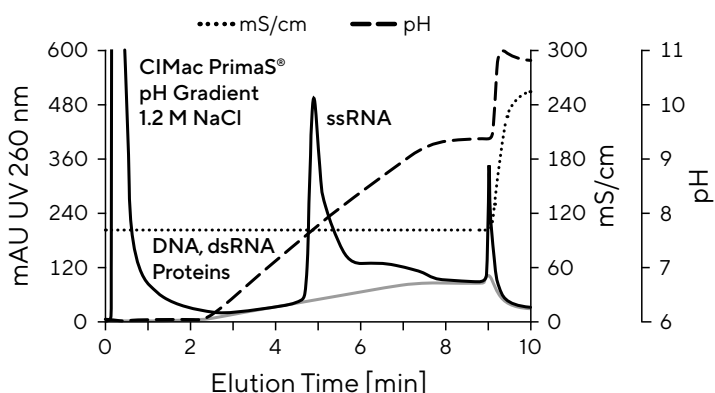
**Figure 1:** Separation of Mixed ssRNA and DNA Ladders in a pH Gradient at 1.2 M NaCl



The conditions shown in Figures 1 and 2 can be used for fast easy purification of research-grade single-stranded mRNA direct from IVT mixtures. The process can be scaled to accommodate any IVT volume on CIMmultus PrimaS<sup>®</sup> monoliths. It can be simplified to step gradient format. A follow-on polishing step by hydrophobic interaction chromatography on CIMmultus<sup>®</sup> C4 HLD is recommended for preparation of clinical quality material. As with PrimaS<sup>®</sup>, double-stranded species do not bind C4 HLD in NaCl, short transcripts elute before complete ssRNA in a descending salt gradient. NaOH is required to elute proteins.

For additional information on the use of PrimaS<sup>®</sup> and other monoliths for analysis and purification of mRNA, contact us at your convenience or obtain a copy of our new book: Purification of Nucleic Acids [1].

**Figure 2:** Fractionation of an IVT Mixture in a pH Gradient at 1.2 M NaCl



## References

- [1] Purification of Nucleic Acids: a handbook for purification of plasmid DNA and mRNA for gene therapy and vaccines, BIA Separations, Ajdovscina, Slovenia, 2020

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