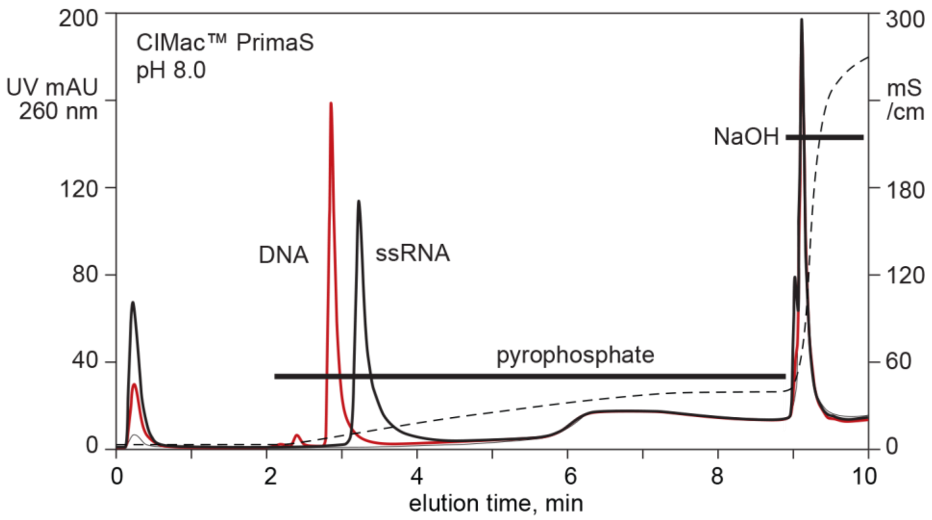
TN0012 Exploring CIMac PrimaS™ for Purity Analysis of mRNA

CIMac PrimaS™ is a new member of BIA's family of high performance chromatography products 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. Large mRNA elutes from CIMac PrimaS at ambient temperature in an ascending pH gradient [1].

New experimental data show that large mRNA can also be eluted from CIMac PrimaS at ambient temperature in a pyrophosphate gradient. Resolution between in vitro transcription (IVT) contaminants and mRNA can be controlled with pH. Figures 1 illustrates the elution characteristics of a DNA ladder (80 bp–10 kbp) and a single-stranded RNA ladder (200 b–6 kb) in a linear gradient to 200 mM sodium pyrophosphate at pH 8.0 (50 mM Tris), followed by cleaning with 0.1 M NaOH + 1 M NaCl.

Figure 1. Separation of ssRNA ladder (black) and DNA ladder (red) at pH 8.0. Overlay of separate chromatograms. The fine black trace marks the buffer baseline by conductivity measuement.



Figures 2 and 3 show the same sample and gradient configuration at pH 7.0 (50 mM Hepes) and pH 6.0 (50 mM MES).

Publication#: TN00012-PrimaSpH-2107-pfr

Figure 2. Separation of ssRNA ladder (black) and DNA ladder (red) at pH 7.0. Overlay of separate chromatograms. The fine black trace marks the buffer baseline by conductivity measurement.

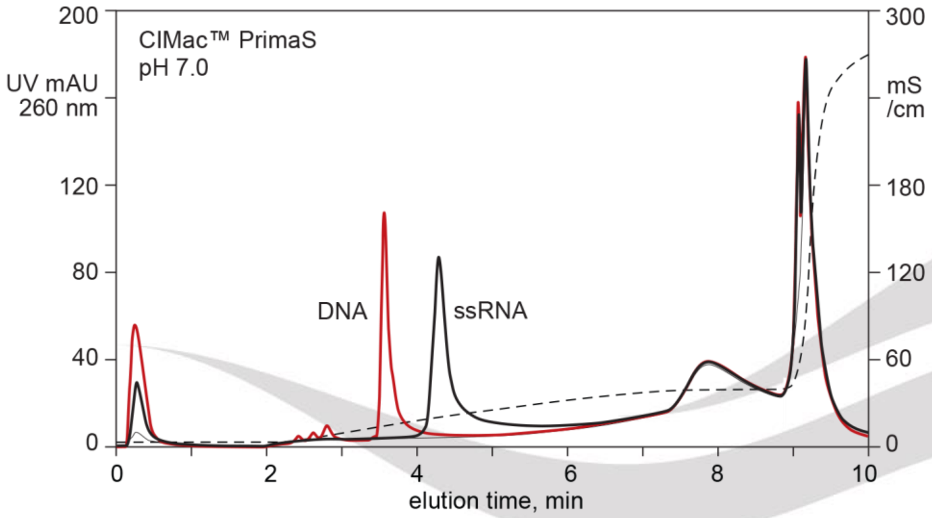
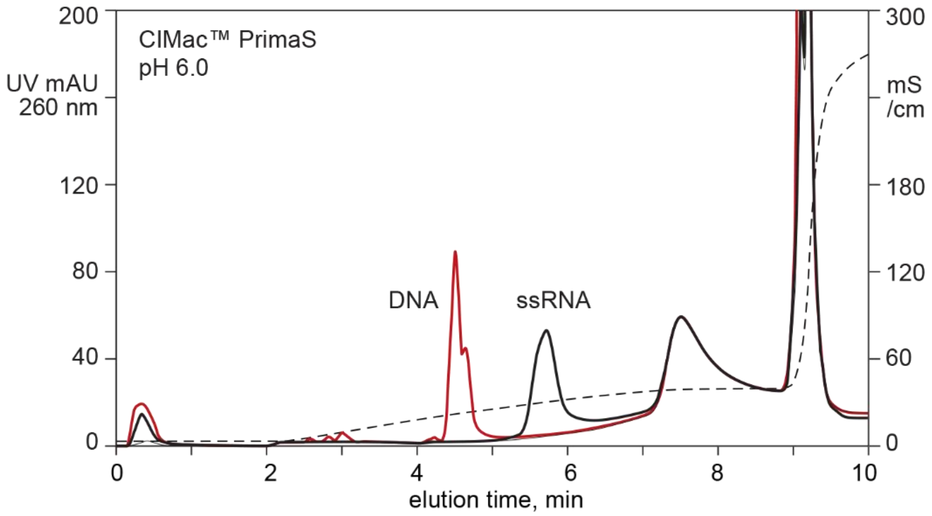


Figure 3. Separation of ssRNA ladder (black) and DNA ladder (red) at pH 6.0. Overlay of separate chromatograms. The fine black trace marks the buffer baseline by conductivity measurement.



Figures 4–6 illustrate fractionation of an IVT mixture at the same pH values. All experiments were conducted on a 100 µL CIMac PrimaS monolith at a flow rate of 10 CV/min (600 CV/h).

Figure 4. Fractionation of an IVT mixture at pH 8.0. The fine black trace marks the buffer baseline by conductivity measurement.

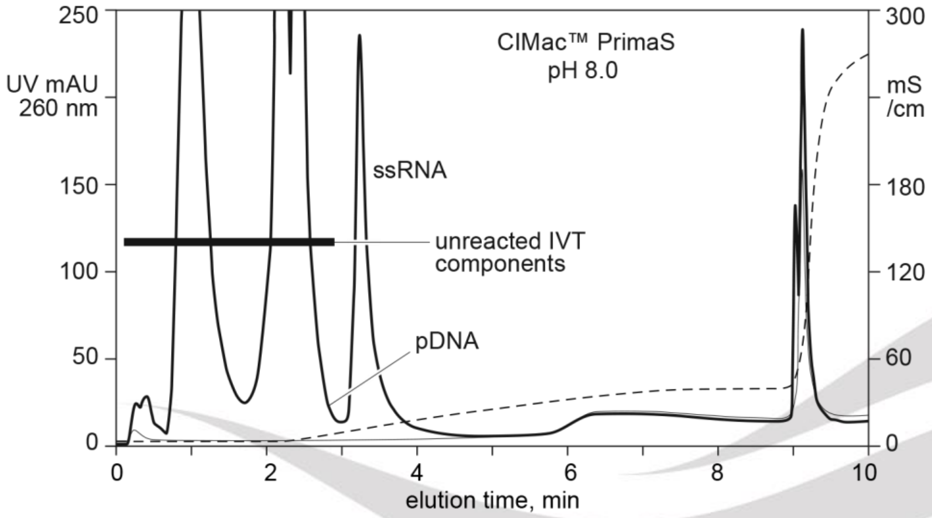
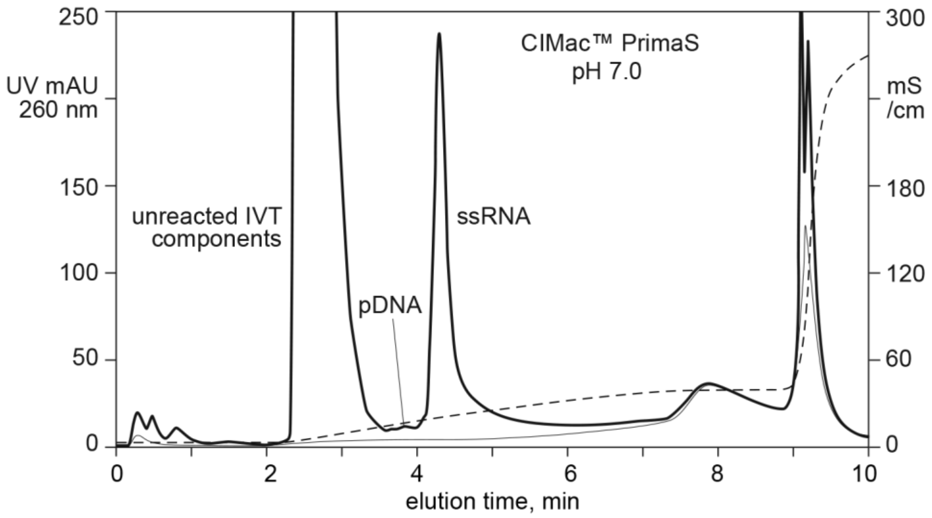
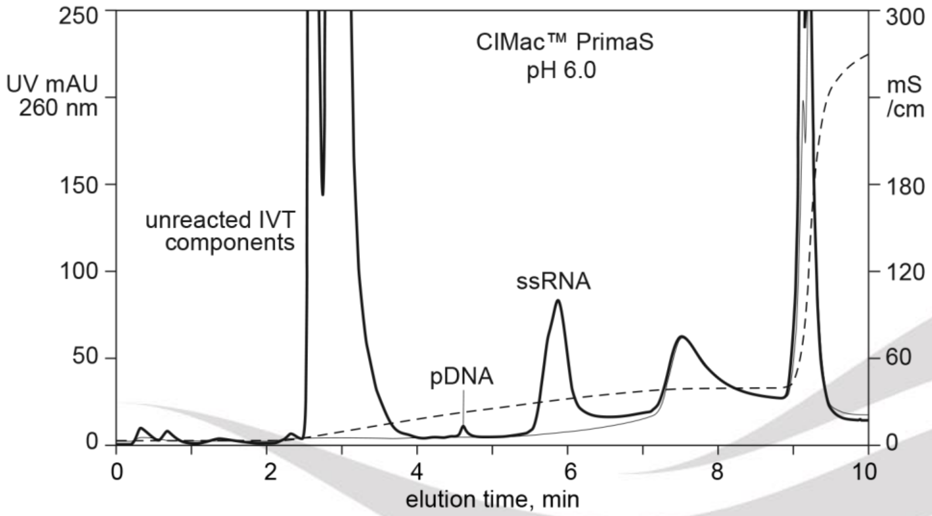


Figure 5. Fractionation of an IVT mixture at pH 7.0. The fine black trace marks the buffer baseline by conductivity measurement.



Publication#: TN00012-PrimaSpH-2107-pfr

Figure 6. Fractionation of an IVT mixture at pH 6.0. The fine black trace marks the buffer baseline by conductivity measurement.



Binding strength for all species increases at lower pH values, shown by their later elution in the pyrophosphate gradient. Resolution also increases at lower pH values. These findings are counterintuitive for anion exchangers and highlight the contribution of hydrogen bonds. RNA has about 20 times more hydrogen bonding sites than charged sites [1]. Hydrogen bonding grows stronger on PrimaS at lower pH values as a function of increasing protonation. The trend continues at lower pH values but stability of mRNA must be kept in mind.

Linear gradient elution is ideal for analysis of mRNA samples because it shows the relationships among all the sample components. For preparative applications, it is usually desirable to eliminate the majority of contaminants in advance. This can done on PrimaS by applying a sodium chloride wash in advance of eluting the mRNA with pyrophosphate or pH. Proteins, DNA, and dsRNA are removed while ssRNA remains bound [1].

For additional information on the use of PrimaS and other monoliths for analysis and purification of mRNA, obtain a copy of our new book: Purification of Nucleic Acids [1].

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: https:www.biaseparations.com/en/products/monolithic-columns/books

Ordering information

Cat No.:	Product description
110.5118-2	CIMac PrimaS™ 0.1 mL Analytical Column (2 µm channels)
311.5118-2	CIMmultus PrimaS™ 1 mL Monolithic Column (2 µm channels)
414.5118-2	CIMmultus PrimaS™ 4 mL Monolithic Column (2 µm channels)
411.5118-2	CIMmultus PrimaS™ 8 mL Monolithic Column (2 µm channels)
614.5118-2	CIMmultus PrimaS™ 40 mL Monolithic Column (2 µm channels)
611.5118-2	CIMmultus PrimaS™ 80 mL Monolithic Column (2 µm channels)
814.5118-2	CIMmultus PrimaS™ 400 mL Monolithic Column (2 µm channels)
811.5118-2	CIMmultus PrimaS™ 800 mL Monolithic Column (2 µm channels)

For cGMP compliant columns, please contact sales@biaseparations.com.

Related products

Cat No.:	Product description
1000-1	Purification of Nucleic Acids printed version (shipping included)
1000-2	Purification of Nucleic Acids pdf
1000-3	Purification of Nucleic Acids epub

For any additional information please contact us:

Tel.: +386 5 9699 500 | sales@biaseparations.com | www.biaseparation.com

Publication#: TN00012-PrimaSpH-2107-pfr