

## Scalable mRNA Purification Using CIMmultus PrimaS® Multimodal Chromatography



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### Introduction

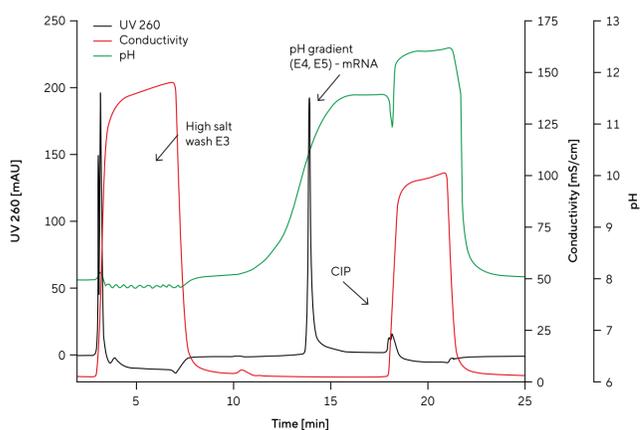
CIMmultus PrimaS® monolithic columns combine multimodal anion exchange | hydrogen bonding properties, binding molecules with a predominantly negative charge. These columns are commonly used as a capture method for the purification of mRNA from in vitro transcription (IVT) reaction mixture with high binding capacity. A high salt wash is used to elute the plasmid and other IVT components from the column without affecting the binding of single-stranded RNA (ssRNA).

### Versatile mRNA Purification Strategies

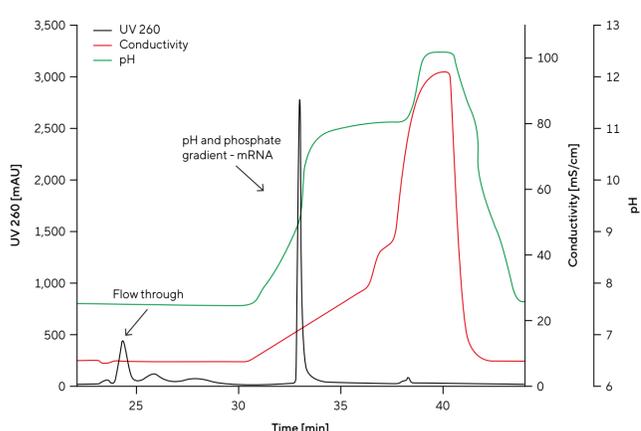
pH Gradient, pH | Phosphate Gradient, pH | Pyrophosphate Gradient

CIMmultus PrimaS® can be used for mRNA purification in multiple modes: (1) pH gradient (pH 8-11; Figure 1), (2) pH gradient combined with phosphate gradient (Figure 2), and (3) pH gradient combined with pyrophosphate gradient (Figure 3).

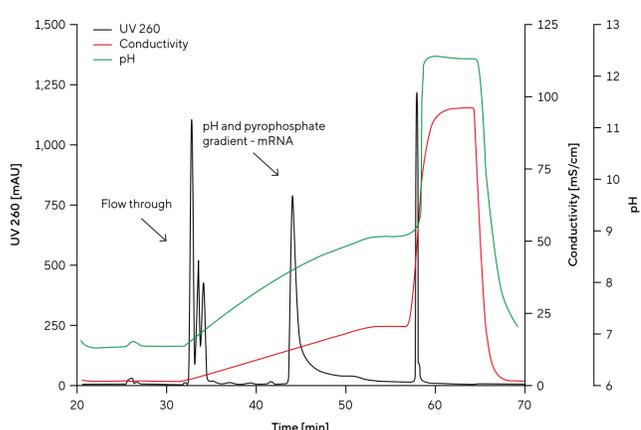
Impurities from the IVT reaction are removed either in a high salt wash step (method 1), flow through (method 2), or in flow-through and the pH gradient (method 3). mRNA elutes in pH gradient. Method 1 (applying a pH gradient; Figure 1) offers the simplest tool for mRNA isolation and achieves more than 90% recovery. mRNA elution occurs at pH 10.5, and the elution fraction is immediately neutralized with an acidic buffer (e.g., KOAc) to prevent mRNA degradation. After neutralization, mRNA is stable even at 37 °C for at least one month. Elution can be performed as a step gradient. Method 3 (Figure 3) achieves mRNA elution at pH 8.5, but the recovery is lower compared to method 1.



**Figure 1:** Isolation of mRNA From IVT Using pH Gradient (pH 8-11). Impurities Are Removed in a High Salt Wash, and Pure mRNA Elutes in the pH Gradient, Achieving a High Recovery



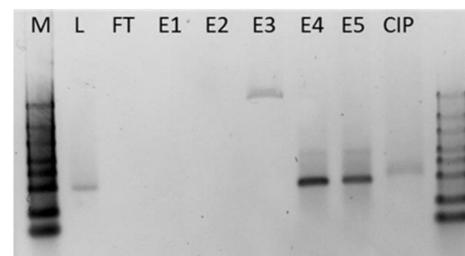
**Figure 2:** Isolation of mRNA From IVT Using pH Gradient (pH 7.5-10.5) | Sodium Phosphate Gradient. Impurities Are Removed in the Flow Through, Achieving Very High Recovery



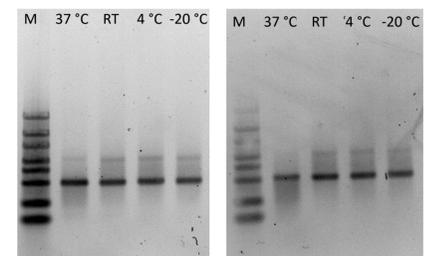
**Figure 3:** Isolation of mRNA From IVT Using pH (pH 7.0-8.5) | Pyrophosphate Gradient. Impurities Are Removed in the Flow Through and Elute Earlier in the pH Gradient, Achieving Lower Recovery

### Stability of mRNA Purified With CIMmultus PrimaS® Column

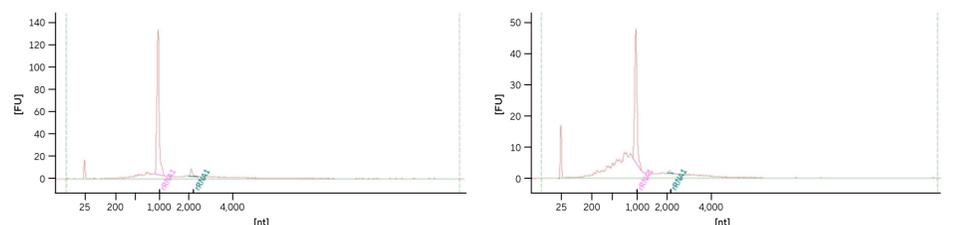
A stability study was performed on mRNA purified from an IVT reaction with CIMmultus PrimaS® using a pH gradient (method 1). mRNA was eluted at pH 10.5 followed by immediate neutralization with 3 M KOAc, pH 5.5. The elution fraction was diluted to 100 ng/mL, and incubated at different temperatures: -20 °C, 4 °C, room temperature, and 37 °C. Aliquots were analyzed after 7 and after 28 days.



**Figure 4:** Agarose Gel Electrophoresis (AGE) Of eGFP mRNA Fractions Purified by CIMmultus PrimaS® (Day 0)



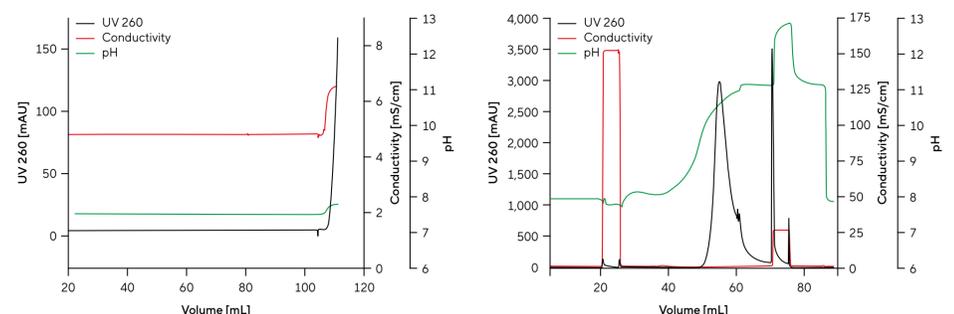
**Figure 5:** AGE of eGFP mRNA Purified by CIMmultus PrimaS® Incubated at -20 °C, 4 °C, Room Temperature (RT), and 37 °C for 7 Days (Left) And 28 Days (Right)



**Figure 6:** Bioanalyzer Trace of mRNA Incubated at Room Temperature (RT; Left) And 37 °C (Right) For 28 Days

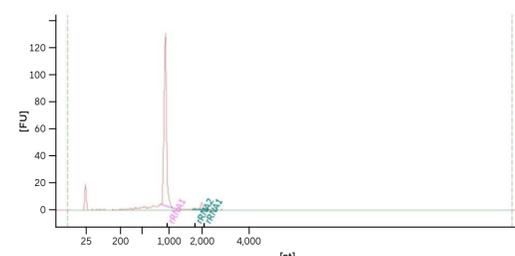
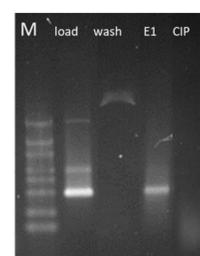
### CIMmultus PrimaS® Has a High Binding Capacity for mRNA

Breakthrough curves were used to determine that the dynamic binding capacity (DBC) of CIMmultus PrimaS® was 6 mg/mL for pre-purified mRNA and up to 5 mg/mL for mRNA from the IVT reaction mixture (eGFP mRNA, 995 nt; Figure 7).



**Figure 7:** Testing the Capacity of CIMmultus PrimaS® for Pre-purified mRNA by Loading Until Breakthrough (Left) Followed by Elution of mRNA Using pH Gradient (Right).

### Confirming the Identity and Purity of Elution Fractions



**Figure 8:** Purity eGFP mRNA Purified by CIMmultus PrimaS®. Left: AGE of CIMmultus PrimaS® Fractions. Wash Fraction Contains Plasmid, E1 Contains mRNA. Right: Bioanalyzer Electropherogram of Neutralized Elution

### Conclusion

- CIMmultus PrimaS® is a tool for simple and quick mRNA isolation, with good recovery, purity, and mRNA stability
- A significant advantage of the CIMmultus PrimaS® is its ability to purify RNA species without polyA tail
- Binding capacity for pre-purified mRNA is ≤6 mg/mL, and for mRNA from IVT is ≤5 mg/mL
- The method includes three important steps: (1) high salt wash step | flow through, to remove IVT components, (2) pH gradient step to elute mRNA (3) cleaning-in-place (CIP) step to remove strongly bound contaminants
- mRNA isolated with CIMmultus PrimaS® is stable for at least a month at 37 °C