SARTORIUS

Method Guide

CIM® PrimaS for mRNA Purification

Convective Interaction Media (CIM®) chromatographic devices are available in different formats, from high throughput 96-well and 24-well plates, analytical chromatographic columns, and scalable purification columns. Chromatographic conditions such as buffer properties (type, pH, conductivity), gradients, or column cleaning may differ between applications. The equipment used for different formats (high throughput, analytical, and preparative devices) offers varying capabilities, such as ability to run gradient elutions. The following instructions can be directly applied to CIMmultus™ preparative columns. Follow format-specific requirements when transferring the conditions outlined here to CIMac™ or CIM® Plate formats.

The ligand in CIM® PrimaS contains a series of hydrogen donors and hydrogen acceptors, combined with a weak anion exchange character. Its hydrogen bonding ability makes its selectivity entirely distinct from traditional anion exchangers. PrimaS purifies large single-stranded mRNA (ssRNA) under aqueous conditions at ambient temperature. Contaminants are removed by means of a hight salt wash, while ssRNA is recovered from the column with a pH gradient (Figure 1) It can be used for purification or analytics of ssRNA, including RNA exceeding 10 kb (such as self-amplifying RNA). In a purification process, it is most often employed for capture of RNA in crude samples such as IVT mixtures.

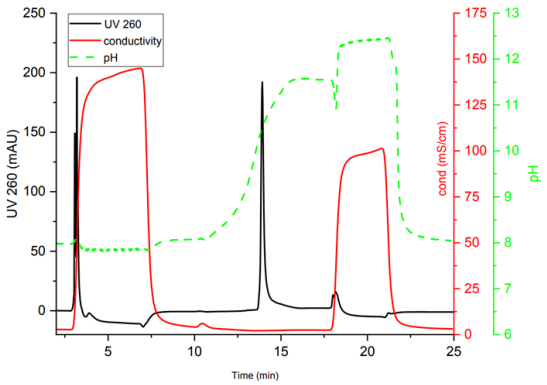

Figure 1: Chromatogram of IVT mixture applied to CIMmultus PrimaS. The impurities elute in the high-salt wash (see conductivity trace), while pure mRNA elutes in the pH gradient (pH 8-11).

For more information, contact
help.BIA@sartorius.com

CIMmultus PrimaS combines anion exchange and hydrogen bonding properties. It binds molecules with predominantly negative charge and repels molecules with a predominantly positive charge. Samples are applied at neutral pH. A high salt wash is used to remove most contaminants, while ssRNA is eluted by increasing pH, which reduces the binding ability of the PrimaS column.

Getting Started

Your column instruction manual can be downloaded by scanning the QR code on the right or by following this hyperlink. CIMmultus columns use a radial flow distribution inside the housing, requiring the column to be connected to the chromatograph with the correct flow direction. Note that some chromatographs have default reverse-flow functions built into their software that can cause the flow direction to be reversed without warning. Make sure this function is disabled before conducting any experiments.

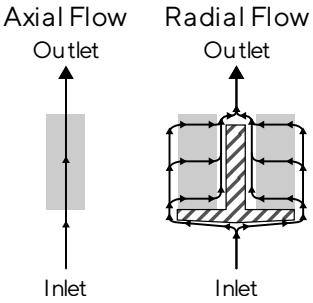

Figure 2: Comparison of axial flow and radial flow distribution within stationary phase of the chromatographic column.

Before applying any sample, prepare the column by removing the storage solution as instructed in the product sheet. It is also recommended to perform a run without sample to provide a baseline against which to compare experimental results. Some buffer components absorb UV, such as EDTA, and some transitions between buffers may create refractive index artefacts that can confuse interpretation of experimental results.

Sample and preparation. CIMmultus PrimaS can be used to process in vitro transcription (IVT) mixtures, including after digestion with DNAse and/or proteinase K. ssRNA resuspended from salt or organic solvent precipitates, or partially purified ssRNA from other purification methods may be evaluated using the same procedure. Avoid freezing IVT mixtures and process the material as soon as possible to prevent precipitation or loss of mRNA. If necessary, particulates can be removed by centrifugation or filtration (0.45 µm) in advance of purification. All work must be carried out in an RNAse-free environment.

Purification of ssRNA with CIMmultus PrimaS

The following guide should provide a good scouting run for further optimisation. See the optimisation and trouble-shooting section for further guidelines.

- Buffer A. Equilibration buffer | Binding buffer. 25 mM Tris, pH 8.
- Buffer B. High-salt wash buffer. 25 mM Tris, 1 M NaCl, pH 8.
- Buffer C. Elution buffer. 25 mM CAPS, pH 11 | 25 mM Glycine, pH 10.5.
- Buffer D. Cleaning buffer. 0.1 M NaOH, 1 M NaCl.
- Neutralization buffer. 3 M potassium acetate, pH 5.5.
- Flow rate. Start with a flow rate of 5 CV/min on CIMmultus 1 mL.

Column equilibration. Wash the column with 10 CV Buffer B, followed by equilibration buffer until output pH and conductivity are the same as the input buffer. Repeat the procedure with buffer A.

Prepare and load sample. Dilute the RNA sample 10-times in buffer A to adjust the pH and to provide binding conditions, then load it on the column. Observe operating pressure during application of large volume samples. Reduce flow rate if necessary to maintain operating pressure within acceptable limits.

Wash1 with buffer A. 5 CV of equilibration buffer. It is not necessary to wait for UV signal to return fully to baseline because trailing contaminants will be eliminated by the subsequent high-salt wash step.

Wash2 with buffer B. Wash the column with 10-20 CV of high-salt wash buffer. Collect the eluting material to analyse for DNA, nucleotides, proteins, and other contaminants.

Wash3 with buffer A. Wash the column with buffer A until UV signal, pH and conductivity are stable.

Elute with buffer C. 50 CV linear gradient to 100% elution buffer C, or a step to 100% elution buffer C.

Eluate neutralization. Neutralise elution fractions immediately after elution with neutralisation buffer. Add 2.5 – 5% volume of the elution fraction volume.

Clean with buffer D. Treatment with at least 10 CV of sanitisation buffer is recommended after every run since it will reveal if a significant amount of material that remains bound to the column at the end of the pH gradient. The contents of the cleaning step may be collected and neutralised upon elution for further analysis. Maintaining a continuous flow during cleaning tends to produce better results since it continually washes foulants out of the column instead of merely hydrolysing them in place. Indications of inadequate cleaning may include a gradual increase of operating pressure over a series of runs, a selectivity shift where a given species elutes earlier or later than in a previous run, and/or the appearance of ghost peaks, where peaks appear during elution despite no sample having been injected.

Column neutralisation. To extend the column lifetime, neutralise the column immediately after cleaning. A 20 CV wash with acetic acid is recommended. Consult the Product Sheet for more information.

Storage. Store the column in 20% ethanol. Take special precautions to avoid following NaOH directly with ethanol as this will form ethoxide radicals that may significantly degrade the ligand in minutes. The column must be at near-neutral pH before introducing ethanol. To store the column, equilibrate it with 10 CV equilibration buffer and then flush with 10 CV of 20 % ethanol.

Optimisation and Troubleshooting

Use the initial scouting chromatogram as a guide for optimizing the composition and duration of the individual steps described above.

Effects of salt. Extensive variation of the high-salt wash is possible, for example using different chaotropic salts at concentrations up to full saturation and addition of different chelating agents. The concentrations given in the protocol are intended as starting points. If lower concentrations provide equivalent purity, they will reduce material expense and simplify buffer preparation. Non-chaotropic salts can be substituted for chaotropic salts but exercise caution when applying high concentrations of salts that precipitate RNA, for example sodium chloride, potassium chloride and lithium chloride. Preliminary experimental results indicate that washing with 1 M sodium chloride clears most of DNA and proteins but may also require an extended wash to restore UV absorbance to baseline.

A workflow simplification can be performed in which the sample is loaded in high salt wash conditions. Salt is added directly to the sample and the column is equilibrated to the high-salt wash buffer. Follow the sample load with a first wash using high-salt buffer, then a second wash using buffer A from above, then elute. This approach may support higher ssRNA binding capacity since it will prevent impurities from competing for binding surface area.

Addition of salt to the pH gradient may affect the pH at which elution occurs. The use of salts that precipitate RNA is acceptable as long as their concentration is maintained well below precipitating levels (Figure 3). Multivalent salts like citrate and EDTA shift the profiles to lower pH values than monovalent salts but also need to be kept at low concentrations. Chaotropic salts tend to give sharper peaks because they maintain RNA solubility. They can be used at any concentration during pH elution but consider that they may interfere with some follow-on chromatography methods.

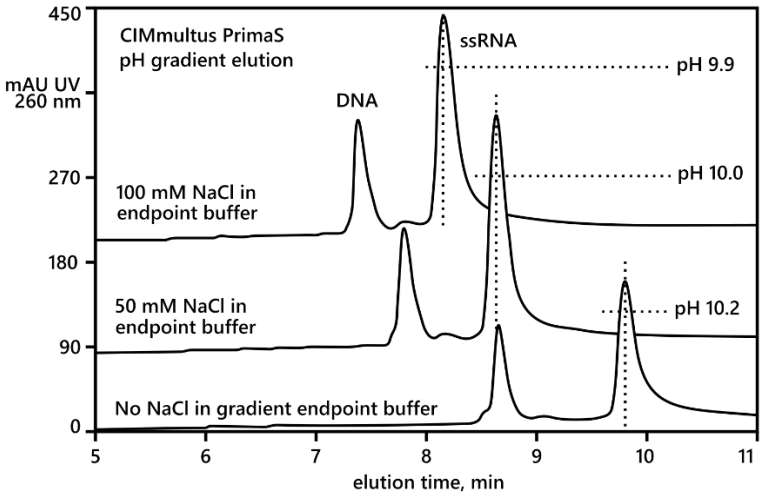

Figure 3. Reduction of elution pH by the presence of salt. Identical sample injections with different elution conditions.

Effects of pH. Variation of the loading sample pH can affect the strength of RNA binding on the column. Lower pH may lead to observation of higher CIP peaks containing RNA. It is recommended to evaluate a pH between 6 and 8 for binding. The linear pH gradient elution can be converted to a step gradient format. In some cases, such as where CIMmultus PrimaS is combined with an orthogonal purification method, it may be practical to elute the ssRNA with a single pH step.

Effects of temperature. CIMmultus PrimaS was designed to avoid the need for elution at elevated temperature but it does not preclude the possibility. Expect elution pH to diminish. This also means that uncontrolled operating temperature may compromise reproducibility.

Effect of flow rate. Different flow rates may be evaluated and may have a small impact on binding of RNA.

Ordering Information

Cat No.	Product Name
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 and 40 L column, please visit www.biaseparations.com or contact sales@biaseparations.com.

FAQ

What is the typical dynamic binding capacity of CIMmultus PrimaS column?

Typical dynamic binding capacity of CIMmultus PrimaS column is in the range of 4-8 mg/mL. Capacity may vary depending on chromatographic conditions, sample properties and degree of method optimisation. The binding capacity scales linearly with the volume of the column.

Can the CIMmultus PrimaS column efficiently remove dsRNA impurities?

Short double-strand RNA (e.g., RNA ladders used in AGE) can often be separated from full length ssRNA. However, dsRNA from IVT is not efficiently removed with the conditions described. If dsRNA impurities removal is the primary objective, it is recommended to use ion pair reversed phase with CIMmultus SDVB.

Is the column reusable? How many times?

Yes, the column is reusable if appropriate cleaning after each run is performed. Column lifetime may vary with sample properties, sample preparation, and column maintenance. The general Cleaning in Place (CIP) procedure is described in the Product Sheet, downloadable from: https://www.biaseparations.com/en/certificates.

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Status: PrimaSinstrRNA-2307-qgh