SARTORIUS

Method Guide

CIM® SDVB 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.

CIM® SDVB is a polystyrene divinylbenzene monolith used for reversed phase (RP) purification of large biologics. It is used in purification of nucleic acids for size fractionation (Figure 1). In addition to length, the degree of base pairing influences selectivity and can enable separation between double-stranded and single-stranded species of similar length. As a preparative tool, CIMmultus SDVB gives its best results as a polishing method. It can be used for fast, low pressure and room temperature purification of RNA, including RNA exceeding 10 kb (such as self-amplifying RNA).

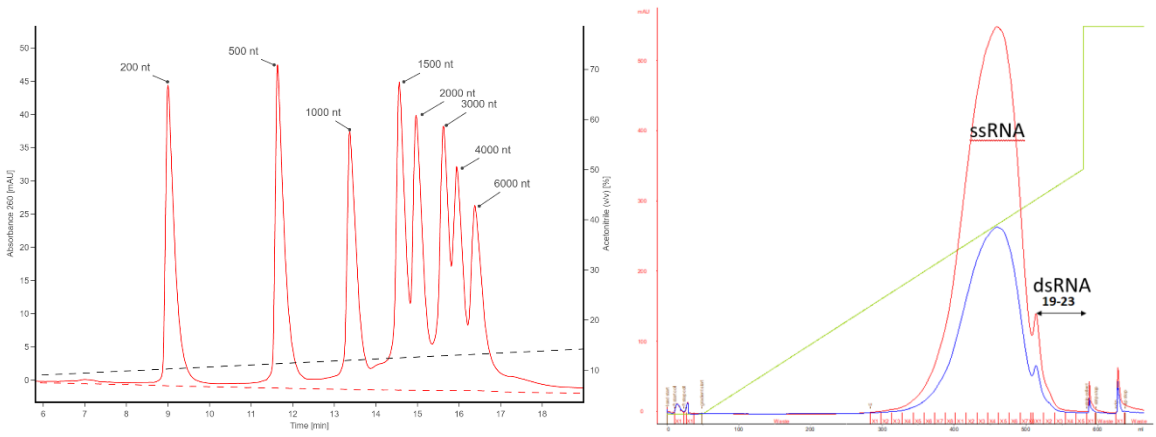

Figure 1: Left: Analytical scale size-fractionation of a 200b-6kb RNA ladder by CIMac SDVB, at 65 °C. Right: Separation between ssRNA and dsRNA of eGFP mRNA using CIMmultus SDVB, room temperature.

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

CIMmultus SDVB is commonly but not exclusively used with an ion-pair reagent. The ion-pair reagent neutralises the negative charge on the RNA backbone to improve binding of RNA or other nucleic acid to the monolith. Elution is achieved by increasing concentration of organic solvent such as acetonitrile.

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.

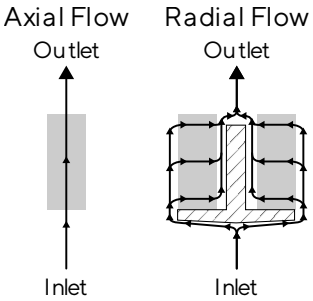

Figure 2: Comparison of axial flow and radial flow distribution within stationary phase of the chromatographic column.

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. It is recommended to always run gradient transitions between aqueous and organic mobile phases, and to avoid step methods.

Sample and preparation. CIMmultus SDVB can be used to increase purity of any sample that contains ssRNA. However, a substantial degree of purification is recommended in advance. Strongly hydrophobic contaminants in IVT mixtures bind very strongly and potentially interfere with overall fractionation performance, especially if large sample volumes are loaded. Intact plasmid DNA will compete with the desired ssRNA for binding capacity and may co-elute to some extent. Dilute the samples with equilibration buffer, at a ratio of at least 4 parts equilibration buffer to 1 part sample. Remove particulates by centrifugation or filtration (0.45 µm) in advance of injection. It is common but not universal practice to pre-incubate the sample to the temperature at which chromatography will be performed.

Purification of ssRNA with CIMmultus SDVB

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/wash buffer. 0.1 M triethylaminoacetate (TEAA), 7.5 % acetonitrile (ACN), pH 7.0.
- **Buffer B**. Elution buffer. 0.1 M TEAA, 25% acetonitrile (ACN), pH 7.0.
- **Buffer C**. Cleaning buffer. 0.1 M TEAA, 90 % ACN.
- **Flow rate.** Start with a flow rate of 5 CV/min on CIMmultus 1 mL.

Column equilibration. Pump equilibration buffer through the column until output pH and conductivity are the same as the input buffer.

Prepare and load sample. Observe operating pressure during application of large volume samples, especially with crude samples like IVT mixtures. Reduce flow rate if necessary to maintain operating pressure within acceptable limits.

Wash with buffer A. 20 CV of equilibration buffer.

Elute with a linear gradient to buffer B. 50 CV to 70% elution buffer. Follow with a 5 CV linear gradient to 100% buffer B and continue at 100% for 10 CV.

Clean with a linear gradient to buffer C. 10 CV gradient, then continue with 10 CV 100% C. It is recommended to include a cleaning step after every run since it will reveal if a significant amount of material remains bound to the column at the end of the elution step. The contents of the cleaning step may be collected and neutralized or buffer exchanged for further analysis. The presence of a low-to-moderate amount of RNA in the cleaning fraction is not necessarily cause for concern since it will tend to represent RNA-contaminant heteroaggregates. A large amount of RNA in the cleaning fraction may indicate a need to raise the percentage of acetonitrile at the end of the primary gradient interval, for example by increasing the percentage of buffer B to 75%, 80%, or higher.

Storage. After flushing Buffer C out of the column, store the column in 20% ethanol. If the column has been cleaned with organic solvents, follow the organic solvent wash with a linear gradient to 20% ethanol.

Optimization and Troubleshooting

Use the initial scouting chromatogram as a guide for optimizing the composition and duration of the individual steps described above. Parameters for optimization include ion-pair reagent type and concentration, slope and range of the organic solvent gradient, temperature, pH.

Gradient configuration. Resolution may be enhanced by decreasing the slope of the gradient. Eluting product concentration may be increased by making the gradient steeper. Once the elution conditions of the desired ssRNA are defined, the gradient may optionally be converted to a step format.

Effects of other organic solvents. An extensive range of organic solvents and selectivity modifiers are known in the field of RPC. Some of these systems may improve purification of RNA. The main concern is that some organic solvents may cause the SDVB polymer matrix to shrink or swell. Be wary of solvents that cause a significant reduction or increase of operating pressure or produce a change in the elution behavior over a series of experiments.

Effect of chelating agent. It is optional to treat the sample with a chelating agent, ideally during a previous purification step. Even low levels of multivalent metal cations may stabilize unnatural product conformations and complexes between ssRNA and contaminants.

Effects of temperature. Increasing temperature increases the intensity of hydrophobic interactions. Higher temperatures will likely increase capacity, but they will also affect selectivity and resolution (Fig. 3). This highlights temperature an important process variable. Uncontrolled operating temperature may compromise reproducibility.

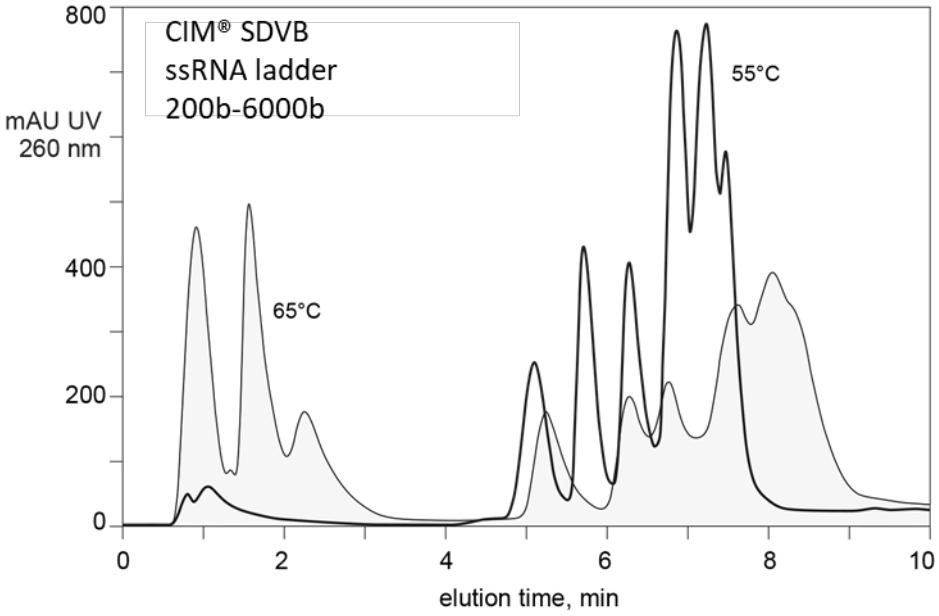

Figure 3. The effect of operating temperature on elution of ssRNA.

Cleaning. Inadequate cleaning may be indicated by a gradual increase of operating pressure over a series of runs, increased tailing during the post-load washing step, a change in the shape of the elution peak, increased contamination of the eluted RNA, and reduced recovery.

Do not use surfactants for cleaning. They bind very strongly to SDVB and interfere with column performance. It may be possible to remove them with propanol-TFA as described above.

Ordering Information

Cat No.	Product Name
311.9001-2	CIMmultus SDVB 1 mL Monolithic Column (2 µm channels)
414. 9001-2	CIMmultus SDVB 4 mL Monolithic Column (2 µm channels)
411. 9001-2	CIMmultus SDVB 8 mL Monolithic Column (2 µm channels)
614. 9001-2	CIMmultus SDVB 40 mL Monolithic Column (2 µm channels)
611. 9001-2	CIMmultus SDVB 80 mL Monolithic Column (2 µm channels)
814. 9001-2	CIMmultus SDVB 400 mL Monolithic Column (2 µm channels)
811. 9001-2	CIMmultus SDVB 800 mL Monolithic Column (2 µm channels)
1014. 9001-2	CIMmultus SDVB 4000 mL Monolithic Column (2 µm channels)
1011. 9001-2	CIMmultus SDVB 8000 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 binding capacity of CIMmultus SDVB column?

Typical dynamic binding capacity of CIMmultus SDVB is in the range of 1-1.5 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 TEAA be replaced by a different reagent?

Tetraethylammonium acetate (TEAA) acts as the ion-pair reagent that binds the mRNA phosphate backbone making the molecule more hydrophobic. Different ion pair reagents can be tested to assess how their hydrophobicity promotes the separation.

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 & Instruction Manual (PSIM), downloadable from: https://www.biaseparations.com/library/product-documentation/.

How to store the column? Can the column be stored in a different solution than EtOH 20%?

Recommended storage solution is 20 % Ethanol, but the column can also be stored in working buffers overnight. More information can be found in the Product Sheet & Instruction Manual (PSIM), downloadable from: https://www.biaseparations.com/library/product-documentation/.

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Status: SDVBinstrRNA-2406-rlt