SARTORIUS

Method Guide

CIM® Swiper for Comprehensive RNA Purification

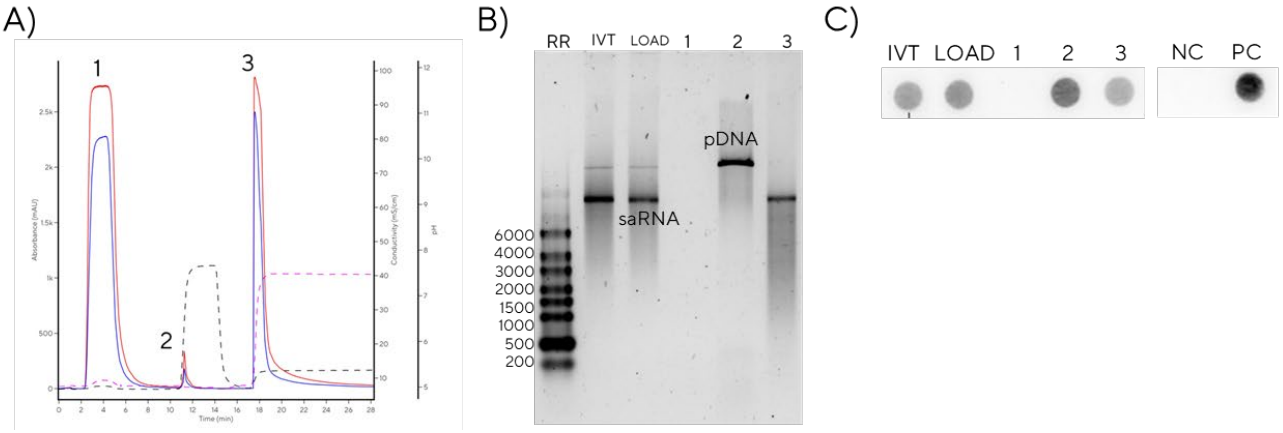
Introduction

CIM Convective Interaction Media® chromatographic devices are available in different formats, from high throughput 96-well and 24-well plates, analytical chromatographic columns, and scalable chromatographic columns for preparative applications in range from 1 mL up to 40 L size. Chromatographic conditions such as buffer composition (including, pH, conductivity), gradients, or column cleaning may differ between applications. 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® Multi-Well Plate formats.

CIMmultus® Swiper is a chromatography column designed for efficient nucleic acid purification in a near-neutral pH range. Based on multimodal weak anion exchange properties, it offers high selectivity between RNA, DNA, and proteins, making it ideal for purification of complex samples. This column efficiently purifies single-stranded RNA of various sizes at neutral pH and ambient temperature, delivering optimal recovery and yield. This makes CIMmultus Swiper suitable for purification of various RNA types, including polyadenylated, non-polyadenylated, and circular RNA, across different sizes and conformations. With optimized ligand properties, the column ensures high recovery under gentle conditions. Its versatility allows for the purification of diverse DNA and RNA modalities, such as mRNA, saRNA, tRNA, circular, and non-coding RNA, without dependence on a poly-A tail.

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

Figure 1: *Isolation of 10 kb saRNA from IVT reaction mixture on CIMmultus® Swiper 1 mL column. A: Preparative chromatogram. 1 - flow-through, 2 – dsDNA wash, 3 – pH step elution. B: AGE analysis of elution fractions. 1 – flow-through, 2 – dsDNA wash, 3 – pH step elution (mRNA is efficiently eluted with a pH gradient). C. dsRNA detection with J2 immunoblot: IVT – IVT reaction mixture, L – Load, 1 – flow-through, 2 – high-salt wash, 3 – pH step elution, NC – negative control (ddH2O), PC – positive control (10 ng dsRNA standard).*

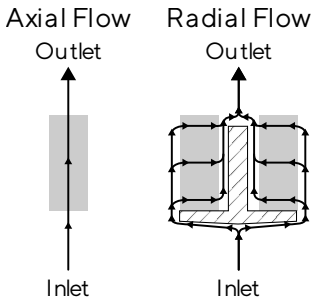


Getting Started

In CIMmultus columns, flow is radial, moving from the outer side through the monolith to the inner side (Figure 2), requiring the column to be connected to the chromatographic system with correct flow direction.

NOTE: *Some chromatographic systems 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 with CIMmultus columns.*

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



Purification of mRNA with CIMmultus Swiper

Sample and preparation: Avoid freezing IVT mixtures and process samples 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 injection. All work must be carried out in an RNAse-free environment. Handle all reagents, materials and equipment in a manner to prevent contamination with RNAses.

Column integrity test: It is highly advised to first perform an integrity test to check the monolith's integrity. Instructions for use (IFU)are available by scanning the QR code on the right or by following this hyperlink.

Flow rate: Chromatography with monoliths ensures flow rate-independent capacity and resolution. When scaling up, adjust the flow rate according to the IFU for specific CIMmultus column size.

Before applying any sample, prepare the column by removing the storage solution as instructed in the product sheet. It is also recommended to clean the column with NaOH (see product sheet) before each use and to first 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.

Buffers and other solutions should be freshly prepared and filtered through a sterile 0.2 µm PES filer. The pH and conductivity of the buffers should be recorded offline and followed by suitable detectors on the chromatograph.

See the optimization and trouble-shooting section for further guidelines.

- **Buffer A**: Equilibration | Load. 50 mM Na-citrate, pH 5.0.
- **Buffer B**. High salt wash buffer. 50 mM Na-citrate, 0.45 M NaCl, pH 5.0 (if target RNA is detected in the wash, adjust NaCl concentration).
- **Buffer C**. Elution buffer. 100 mM Na-phosphate, pH 7.5.
- **Buffer D**. Cleaning buffer. 0.1 M NaOH + 1 M NaCl. Limit contact time with buffer D to 15 min per standard cleaning.
- **Buffer E**. Pre-storage buffer. 25 mM Na-phosphate, pH 2.5.
- **Buffer F.** Storage solution. 20 % EtOH in 20 mM Na-phosphate, pH 2.5.
- **Sample dilution buffer.** 50 mM Na-citrate, pH 5.0.
- **Flow rate.** Start with a flow rate of 2 CV/min.

Column preparation and equilibration.
Step 1. Leave the column and mobile phases to equilibrate to working temperature.
Step 2. Connect the column by the inlet first, followed by the outlet. Ensure that flow will be applied in the direction of the arrow on the housing (applies to radial CIMmultus units).
Step 3. Wash the column with the following steps:
- 5 CV ddH_2O to flush the storage solution from the column
- 10 CV 0.1 M NaOH + 1 M NaCl
- 10 CV buffer B or until pH and conductivity at the column outlet reach the buffer specification
- 10 CV buffer A or until pH and conductivity at the column outlet reach the buffer specification

Before sample application, a blank run should be performed to detect any potential buffer peaks, baseline drifting, or other artefacts on the chromatogram. Buffer peaks are expected at step transitions due to changes in refractive indices. To perform a blank run, follow the instructions below without loading the sample.

Sample and preparation. A freshly prepared IVT reaction mixture should be diluted at least 2- to 5-times with buffer A to provide pH buffering and binding conditions. Dilution factor can be optimised before scale-up. Alternatively, a more concentrated sample dilution buffer (e.g. 100 mM Na-citrate, pH 5.0) can be implemented for sample preparation.

Starting method for mRNA purification from IVT reaction mixture

The expected binding capacity of CIMmultus Swiper for mRNA in buffer A is ~ 2 mg/mL. Binding capacity may vary based on mRNA size and should be determined for each individual construct in a given matrix. Use the appropriate column size for your expected mRNA amount.

Step 1. Load the sample to the column at a flow rate of 0.5-5 CV/min. Observe the operating pressure during application of sample and stop the loading if the pressure shows an exponential increase.
Step 2. Monitor UV absorbance at 260 nm and collect the flow-through fraction.
Step 3. Wash with 2-10 CV buffer A to remove unbound material until UV signal returns to baseline.
Step 4. Wash with 2-10 CV buffer B to remove dsDNA and analyze fraction for RNA and contaminants.
Step 5. Wash with 2-5 CV buffer A to lower conductivity.
Step 6. Elute mRNA with step gradient of 5-10 CV buffer C (optionally, employ a linear gradient from buffer A to buffer C, e.g. over 20 CV).

Column cleaning and storage. If column is to be reused, it should be stored appropriately to enable a reproducible purification process.
Use the following procedure:
Step 1. Clean the column with 10 CV buffer D (contact time up to 15 min). Recording UV during cleaning can provide information on strongly bound material. Some mRNAs may be detected in the cleaning fraction.
Step 2. Wash with at least 10 CV buffer B or until pH at the column outlet reaches the buffer pH.
Step 3. Wash with at least 10 CV buffer E or until pH and conductivity are stable.
Step 4. Wash with 10 CV buffer F or until pH and conductivity are stable.

The cleaning step implemented in the procedure should provide a reproducible purification process. A more thorough cleaning can be implemented if run to run pressure increase is observed by extending the contact time with the cleaning solution (solution D) and/or increasing NaOH concentration in the cleaning solution up to 0.5 M (e.g. 0.5 M NaOH + 2 M NaCl).

Optimization and Troubleshooting

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

Effects of pH. Electrostatic interactions become stronger with decreasing pH due to increasing protonation of ligand. This means that nucleic acids will bind more strongly with increasingly acidic pH. Increasing pH should have the opposite effect, which may be useful to enhance recovery during elution.

Effects of salts. Optimization of salt concentration for washing may be required for each mRNA to achieve selective removal of dsDNA and prevent mRNA loss. Salt concentration in wash buffer may need to be adjusted also when changing pH. The most common salt is NaCl and a typical range of 0.2 - 1 M NaCl should be tested for washing.

Ordering Information

Cat No.	Product Name
311.5122-2	CIMmultus® Swiper 1 mL Monolithic Column (2 µm channels)
411.5122-2	CIMmultus® Swiper 8 mL Monolithic Column (2 µm channels)
414.5122-2	CIMmultus® Swiper 4 mL Monolithic Column (2 µm channels)
611.5122-2	CIMmultus® Swiper 80 mL Monolithic Column (2 µm channels)
614.5122-2	CIMmultus® Swiper 40 mL Monolithic Column (2 µm channels)
811.5122-2	CIMmultus® Swiper 800 mL Monolithic Column (2 µm channels)
814.5122-2	CIMmultus® Swiper 400 mL Monolithic Column (2 µm channels)

For cGMP compliant columns please visit www.biaseparations.com or contact sales@biaseparations.com.

FAQ

What is the typical dynamic binding capacity of CIMmultus Swiper column?
Typical dynamic binding capacity of CIMmultus Swiper for mRNA is ~2 mg/mL. With optimization of binding conditions capacity can be increased. Capacity may vary depending on chromatographic conditions, sample properties and degree of method optimisation.

What operating flow rate do you recommend for each column size?
The minimum and maximum flow rate for each column is defined in the Instructions for Use, downloadable from: https://www.biaseparations.com/en/certificates.

Is the column reusable?
Yes, the column is reusable if appropriate cleaning and storage after each run is performed.

Can the CIMmultus Swiper column efficiently remove dsRNA impurities?
CIMmultus Swiper is a multimodal chromatography monolith, combining anion and hydrogen bonding interactions. It is designed to selectively capture RNA molecules, while double stranded species (e.g. dsDNA) are removed in the high salt wash. If removal of dsRNA impurities is the primary objective, it is recommended to use ion pair reversed phase chromatographic approach with CIMmultus SDVB.

Can an enzyme-treated sample be loaded onto the CIMmultus Swiper column?
Use of enzymes does not affect the performance of the column. CIMmultus Swiper can be used to process in vitro transcription (IVT) mixtures and nucleic acid samples after treatments with different enzymes.

Can the CIMmultus Swiper column be used to purify DNA?
CIMmultus Swiper can be used to purify DNA, but chromatographic conditions may need be adjusted. We recommend testing salt (e.g. 0-1 M NaCl) gradients at acidic pH (5-6) and pH gradients (e.g. from pH 5 to 7.5) to identify optimal starting strategy and conditions for your sample.

How to store the column?
Recommended storage solution is 20 % EtOH in 20 mM Na-phosphate, pH 2.5. Storing the column at different conditions may influence column performance and lifetime.

More information can be found in the Instructions for Use, downloadable from:
https://www.biaseparations.com/en/certificates.

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