SARTORIUS

Method Guide

CIM® Oligo dT18 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 CIMmultusŏ preparative columns. Follow format-specific requirements when transferring the conditions outlined here to CIMacŏ or CIM® Plate formats.

The ligand in CIM® Oligo dT18 is an 18-mer deoxythymidine chain covalently immobilised on the monolith with a carbon linker. RNA with a poly(A) tail binds on the ligand by hybridisation between adenine (A) bases on the RNA tail and deoxythymine (T) bases of the ligand. Species which do not contain a poly(A) tail do not bind on the column and are found in the unbound (flow through) fraction. CIM® Oligo dT18 can be used for purification or analytics or ssRNA, including RNA exceeding 10 kb (self-amplifying RNA). In a purification process, it is most often employed for capture of RNA in crude samples such as IVT reaction mixtures.

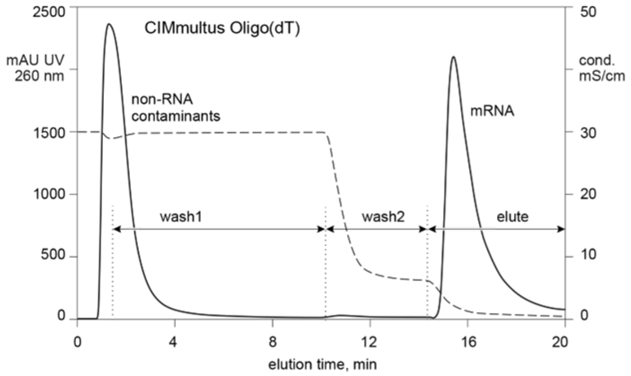

Figure 1: Chromatogram of IVT mixture applied to CIMmultus Oligo dT18. Buffers and conditions as described below

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

The sample is applied at elevated ionic strength to suppress charge repulsion between the phosphatidic acid residues on the ligand and the target RNA. RNA is recovered by reducing ionic strength, which restores charge repulsion sufficiently to dissociate the hydrogen bonds.

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.

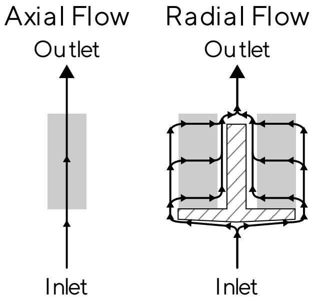

Figure 2: Comparison of axial flow and radial flow distribution within stationary phase of the chromatographic column.

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.

Column variants. The column is available with two different linkers: 6-carbon (C6) and 12-carbon (C12) linker. No differences in performance are expected but may be further evaluated if desired. The recommended default choice is C12.

Sample and preparation. CIMmultus Oligo dT 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 samples as soon as possible to prevent precipitation or loss of mRNA. Sample pH should be roughly neutral and contain a suitable concentration of salt for binding. 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.

Purification of ssRNA with CIMmultus Oligo dT

The following guide anticipates an RNA molecule stable in 1.25 M NaCl. See the optimisation and trouble-shooting section for further guidelines.

- **Buffer A**. Equilibration | High salt wash buffer. 50 mM sodium phosphate, 1.25 M sodium chloride, 2 mM EDTA, pH 7.4.
- **Buffer B**. Low salt wash buffer. 50 mM sodium phosphate, 2 mM EDTA, pH 7.4.
- **Buffer C**. Elution buffer. Deionized water.
- **Buffer D**. Cleaning buffer. 0.5 M NaOH.
- **Sample dilution buffer** (example given for 1.25 M loading NaCl concentration). 50 mM sodium phosphate, 2 mM EDTA, 2.5 M NaCl, pH 7.4.
- **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. Dilute the mRNA sample 1:1 with sample dilution buffer or 10:1 with buffer A to adjust salt concentration. Load the sample on the column. Observe operating pressure during application of large volume samples. Reduce flow rate if necessary to maintain operating pressure within acceptable limits.

High salt wash with buffer A. Wash to return UV signal to baseline. Wait at least 5 CV after the UV signal is stable.

Low salt wash with buffer B. Wash to elute non-specifically and weakly bound contaminants from the column.

Elute with buffer C. Collect the elution peak until UV absorbance returns to baseline.

Clean with buffer D. This step will elute material which may remain bound. A short cleaning is enough to reveal a UV signal on the chromatogram. For cleaning, a contact time of up to 30 minutes is recommended. The contents of the cleaning step may be collected and neutralised | buffer exchanged for further analysis. The presence of moderate amounts of RNA in the cleaning fraction is not necessarily cause for concern since it will tend to represent RNA-contaminant aggregates.

Column neutralisation. Follow cleaning solution immediately with Buffer A for neutralisation. Afterwards, wash with 10 CV of deionised water.

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.

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 pH. Hydrogen bonding becomes stronger with decreasing pH due to increasing protonation of biomolecules. This means that RNA will bind more strongly with increasingly acidic pH. It also creates a potential risk of promoting non-specific binding by contaminants and depressing RNA recovery during elution. Increasing pH should have the opposite effect, which may be useful to enhance recovery during elution.

Effects of flow rate. A way to achieve greater dynamic binding capacity of the column it is to increase residence time. Extending mRNA-monolith contact time enhances hybridization by allowing the mRNA polyA tail enough time to align properly to the oligo dT. Evaluate flow rates between 0.5 and 10 CV/min. See: Development and scale-up of oligo-dT monolithic chromatographic column for mRNA capture through understanding of base-pairing interactions - ScienceDirect.

Effects of salts. Optimization of salt concentration for loading and washing may be required for each mRNA to achieve optimal binding to the column and selective elution. The most common salt is NaCl and a range of 0.25 - 1.5 M NaCl should be tested to improve dynamic binding capacity. Additives such as Gu-HCl and Mg2+ can be added to NaCl-containing binding buffer to improve binding capacity. Gu-HCl can be also used in place of NaCl. We presume that the higher chaotropicity of Gu-HCl compared with NaCl enables stronger binding of mRNA to the oligo(dT) solid support. Note that with Gu-HCl concentrations over 1M in combination with mRNA present above 0.1 mg/mL precipitation can occur.

Experimentation with different species of salts and concentrations can be beneficial but it is important to keep in mind that they have different effects on the chemical mechanisms that control binding and elution: electrostatic repulsion and hydrogen bonding. It is also important to keep a third variable in perspective: many salts precipitate RNA.

Neutral salts like sodium chloride and potassium chloride have a strong effect on electrostatic repulsion but they have a relatively weak effect on hydrogen bonding. This implies that sodium chloride should be applicable at very high concentrations, with a hypothetical benefit of helping to suppress non-specific binding by contaminants. This is impractical however because the RNA precipitates they form would likely interfere with flow through the column.

Salts comprising multivalent anions like phosphate, sulphate, and citrate have intermediate characteristics. Mole per mole, they more effectively suppress electrostatic interactions than monovalent salts. They are also more effective hydrogen donor-acceptors than neutral salts because of the oxygen residues they carry. On the negative side, they tend to promote associations among biomolecules, leading to formation of complexes and precipitates, so their concentrations should be kept low. A more subtle issue is that they are often highly contaminated with multivalent metal cations that can alter the conformation of ssRNA and promote formation of metal-bridge associations with dsRNA, DNA, and proteins. Phosphate buffers may also be contaminated with pyrophosphates (diphosphates). In living systems, pyrophosphates form precipitates with calcium that can lead to adverse health consequences. Phosphate buffers are typically not labeled to indicate pyrophosphate content. Anhydrous phosphate buffers carry the highest proportion of pyrophosphates because of the high temperature used to drive off hydration water. If phosphates are used at all, it is recommended to use hydrated phosphates.

Chelating salts have special capabilities for enhancing the performance of CIMmultus Oligo dT. They dissociate metal-bridge complexes among multivalent metal cations, nucleic acids, and proteins. In other words, they tend to disaggregate large non-specific complexes between ssRNA and contaminants. They are most effective when present in sample and in the equilibration/wash buffer. Consider concentrations in the range of 5–10 mM.

Effects of other additives. Other additives can be evaluated as loading conditions, specific wash steps, or to achieve different elution formulation. The following application note evaluates binding conditions using design of experiment: Screening binding conditions on CIM Oligo dT 96-well plate to optimize dynamic binding capacity - Sartorius BIA Separations.

Effects of temperature. Increasing temperature weakens hydrogen bonding. RNA samples are often exposed to elevated temperature in advance of being applied to chromatography columns and sometimes during chromatography. Higher temperature during sample application risks reducing binding capacity. Higher temperature during elution may improve RNA recovery and cause it to elute in a sharper peak. This is also a warning that uncontrolled operating temperature may compromise reproducibility.

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.

Sample precipitation. Higher salt concentrations promote binding but may also induce more precipitation. Optimisation of salt concentration can prevent precipitation. Reducing the contact time between high salt conditions and sample may allow the use of higher salt concentrations while also preventing precipitation. Use of inline dilution for sample application can further reduce the contact time and allow the use of higher salt concentrations without precipitation.

Further reading

- Development and scale-up of oligo-dT monolithic chromatographic column for mRNA capture through understanding of base-pairing interactions - ScienceDirect
- Screening binding conditions on CIM Oligo dT 96-well plate to optimize dynamic binding capacity - Sartorius BIA Separations

Ordering Information

Cat No.	Product Name
311.1219-2	CIMmultusŏ Oligo dT18 1 mL Monolithic Column (C12 Linker) (2 µm channels)
414.1219-2	CIMmultusŏ Oligo dT18 4 mL Monolithic Column (C12 Linker) (2 µm channels)
411.1219-2	CIMmultusŏ Oligo dT18 8 mL Monolithic Column (C12 Linker) (2 µm channels)
614.1219-2	CIMmultusŏ Oligo dT18 40 mL Monolithic Column (C12 Linker) (2 µm channels)
611.1219-2	CIMmultusŏ Oligo dT18 80 mL Monolithic Column (C12 Linker) (2 µm channels)
814.1219-2	CIMmultusŏ Oligo dT18 400 mL Monolithic Column (C12 Linker) (2 µm channels)
811.1219-2	CIMmultusŏ Oligo dT18 800 mL Monolithic Column (C12 Linker) (2 µm channels)
1014.1219-2	CIMmultusŏ Oligo dT18 4000 mL Monolithic Column (C12 Linker) (2 µm channels)
1011.1219-2	CIMmultusŏ Oligo dT18 8000 mL Monolithic Column (C12 Linker) (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 Oligo dT column?

Typical dynamic binding capacity of CIMmultus Oligo dT is in the range of 2-4 mg/mL when NaCl is used as loading salt. With optimization of binding conditions capacity can be increased. 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.

What operating flow rate do you recommend for each column size?

The flow rate can affect the binding capacity of the column, with lower flow rates providing an advantage. See "Development and scale-up of oligo-dT monolithic chromatographic column for mRNA capture through understanding of base-pairing interactions" in Further reading for more detailed information. The minimum and maximum flow rate for each column is defined in the Product Sheet, downloadable from: https://www.biaseparations.com/en/certificates.

What is the difference between 6-carbon (C6) and 12-carbon (C12) linkers?

C6 and C12 refer to the length of the carbon chain which links the dT(18) to the monolith matrix. Purification performance is comparable but can be further evaluated if desired. CIMmultus Oligo dT with the C12 Linker is the starting column of choice.

Is the column reusable? How many times?

Yes, the column is reusable if appropriate cleaning after each run is performed. Testing showed stability of the ligand up to at least 40 cycles of cleaning in place (CIP) of 30 minutes each. Typical lifetime exceeds this value.

Can the CIMmultus Oligo dT column efficiently remove dsRNA impurities?

CIMmultus Oligo dT is a hybridization-affinity chromatography monolith which binds RNA with a poly-A tail, while species lacking a poly-A sequence, including dsRNA, flow through the column. If dsRNA impurities removal is the primary objective, it is recommended to use ion pair reversed phase with CIMmultus SDVB.

Can a DNAse-treated sample be loaded onto the Oligo dT column?

Use of DNAse does not affect the performance of the column. CIMmultus Oligo dT can be used to process in vitro transcription (IVT) mixtures, including after digestion with DNAse and/or proteinase K.

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, downloadable from: https://www.biaseparations.com/library/product-documentation/.

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
www.sartorius.com

Status: OdTinstr-2406-cfz