Instructions for Use

CIMac Oligo dT18 0.1 mL Analytical Column (C12 Linker) (2 µm channels)

CIM Convective Interaction Media®
110.1219-2

SARTORIUS

Contents

1. About These Instructions for Use

These instructions are part of the device. They apply to the device product number indicated on the cover page.

1.1. Accompanying Documents

In addition to these instructions, the following supporting documents may be consulted.

Guideline: Optimisation of LC system for analytical work	
Sample starting conditions for purification or analytics with Oligo dT	

2. Safety

⚠WARNING
Denotes a hazard that may result in death or severe injury if it is not avoided.

⚠CAUTION
Denotes a hazard that may result in moderate or minor injury if it is not avoided.

NOTICE
Denotes a hazard that may result in property damage if it is not avoided.

2.1. Intended use

CIMac Analytical Monoliths are high performance chromatography devices for rapid high-resolution fractionation of complex biological samples. The stationary phase is polymerised as a monolith with homogeneous channel size and surface chemistry. Each unit is mounted in a precision engineered stainless steel housing to allow easy connection to any HPLC system.

Oligo dT analytical columns are primarily intended for fast and efficient analysis of messenger RNA with a poly-adenylated tail, from various sources. The following information is provided to ensure proper product care and optimal product performance.

2.2. Safety Note

Follow the guidelines in this Instructions for Use. Improper use may result in malfunction, personal injury, or damage of the product or material. Follow safety instructions, wear gloves, safety glasses, and a lab coat during operation.

3. Technical Data

Column chemistry	Oligo dT18 coupled to CDI-activated matrix, C12 Linker
Channel radius	1050 nm (950 nm – 1150 nm)
Support matrix	Poly(glycidyl methacrylate -co- ethylene dimethacrylate)
Monolith dimensions	Diameter: 5.2 mm; length: 4.95 mm; bed volume (CV): 0.1 mL
Connector	10-32 UNF coned port, 1/16'' OD tubing connection
Ligand density	> 0.5 mg Oligo dT/mL wet support
Operating flow rates	0.2 – 3 mL/min (1 – 15 cm/min; 2 – 30 CV/min)
Maximum pressure	15 MPa, 150 bar, 2175 psi
Operating temperature	4 °C (39 °F) to 30 °C (86 °F)
Chemical stability	All commonly used aqueous buffers, sodium hydroxide (short term up to 0.5 M, see cleaning guidelines), 6 M guanidine hydrochloride, 12 M guanidine thiocyanate, 10 M urea, 20 % ethanol.
Recommended pH	Working range 2-10, Cleaning in place 2–13
Storage conditions	2 °C (36 °F) to 25 °C (77 °F); 20 % ethanol
Shelf life	3 years

4. Installation

Remove the product from its shipping box or crate and place on a flat surface. Carefully inspect the product for any damage that may have occurred during shipping. Immediately report any such damage to your vendor and the courier. The product is shipped in the designated storage solution at ambient temperature and should be stored upon receiving as stated under Technical Data.

NOTICE
Do not store the product below 0 °C (32 °F).

5. Getting Started

Use the product per these guidelines. Improper use may result in malfunction, personal injury, or damage of the product or material. Follow general safety instructions for laboratory work.

⚠CAUTION
Set the pressure relief valve of the system (pump) to the value indicated in the table Technical Data.

NOTICE
The column should be equilibrated to working temperature for optimal results. Allow sufficient time for the column to reach working temperature.

Setting up the HPLC system is a crucial factor in achieving optimal performance from CIMac™ Analytical Columns. The following suggestions should be considered:

Capillaries: The inner diameter of the capillaries strongly affects the peak shape. Using capillaries with smaller diameter will result in sharper peaks.

Backpressure: Check the back pressure of the system at a flow rate up to 2 mL/min higher than your working flow rate. Ensure that the back pressure of the system without the column stays at least 10 bar (1 MPa) below the maximum allowed pressure on the column (see Technical Data). Adjust the pressure relief valve accordingly.

Detector: For optimal detector sensitivity set the detector response time to the lowest possible value – for most UV detectors this value is 0.1 s.

Acquisition rate: The acquisition rate depends on the analysis time. A typical analysis time in the case of CIMac™ Analytical Columns is less than 15 min. Data acquisition rate of 5 to 10 Hz is recommended.

Flow rate: Typical analysis flow rates are 0.2–2 mL/min. For flow rate properties of the column see Technical Data.

5.1. General Recommendations

The following are general guidelines to consider when working with chromatography. The guidelines may not apply to specific column chemistry or sample properties.

- Treat loading material appropriately (e.g. pre-treat, filter, concentrate / dilute, etc.). For more details, please refer to the Guideline 'Pre-treatment of complex biological samples before column purification and regeneration procedures for columns with increased back pressure' (biaseparations.com/en/library/guidelines).
- Always use freshly prepared mobile phases, filtered through 0.2 µm filter, compatible with mobile phases.
- Air bubbles will not disturb the stationary phase and can be washed out of the column. However, drying the monolith risks damaging the stationary phase.
- Surfactants can improve recoveries in virus purification. Non-ionic surfactants will not interact with ion exchange chromatography media. Non-UV-absorbing (at working wavelengths) surfactants will improve the baseline signal.
- Ensure all components of the system used are compatible with the working solutions (e.g. sodium hydroxide, organic solvents, high salt concentrations, etc).

NOTICE
Always ensure mobile phases are compatible before mixing them or applying consecutively on the column. Examples of in-compatible buffers are: magnesium ion-containing buffers and sodium hydroxide (forms precipitate), acetonitrile and sodium hydroxide (forms ammonia and acetate), ammonium acetate and sodium hydroxide (potential formation of explosive atmosphere), ethanol and sodium hydroxide (forms ethoxides). Wash the column with water or another compatible solution when using two incompatible solutions consecutively.

5.2. Buffer Selection

Oligo dT binds poly-adenylated RNA by hydrogen bonding interaction between the nucleotide residues in the presence of salt. Salts suppress negative charge repulsion between RNA and the ligand and allow formation of a stable hybrid. Detailed process optimisation parameters can be found in the Accompanying Documents, simple guidelines are outlined here.

The product is compatible with commonly used biological buffers (Tris, BTP, HEPES, etc). Addition of EDTA (up to 20 mM) in all buffers will minimise non-specific effects of multivalent metal cations and is recommended.

Parameters which change the protonation of biomolecules will affect the retention of the sample. A neutral pH is a suitable starting point. Lower pH increases the protonation of nucleotide residues, which results in stronger binding. pH is temperature dependent, and changes in binding or elution performance can be expected without adequate temperature control.

Any salt that precipitates RNA will promote binding. Neutral salts (e.g. sodium, potassium chloride or acetate) are preferred over kosmotropic salts (phosphates, sulfates), which may induce non-specific associations and affect the contaminant level in the product. Excess binding salt may increase binding capacity but it risks non-specific binding and precipitation of RNA. The salt type and concentration can be evaluated experimentally. As a starting point, a range of 250 mM - 1.5 M NaCl can be tested.

6. Operating the Column

6.1. Connecting the Column

Connect the column to the system in the following order:

1. Carefully remove the blind fitting on one side and connect the inlet tubing to the column.
2. Carefully remove the blind fitting on the opposite side and connect the outlet tubing to the column.

The column can be disconnected from the system by reversing the above steps.

Note: The flow path inside the housing is symmetrical, and analysis can be performed in both directions.
Note: It is recommended to apply flow in reverse direction during column cleaning to displace any debris or particles accumulated on the frit of the column.

6.2. Equilibration

The column should be equilibrated before starting with sample analysis, and after column cleaning. Equilibrating the

column will ensure robust and consistent analytical results. Equilibration after cleaning is particularly important for ion exchange columns to replace the counter-ion at its surface. The column may be equilibrated as follows:

1. Remove any storage or cleaning solution by washing with 10 CV of deionised water. **Note:** It is useful to flow the first few CV directly into waste without going through the detector cell. This will remove any air bubbles that may affect the detector cells.
2. Wash the column with at least 20 CV of binding mobile phase.
3. Wash the column with at least 20 CV of the eluting mobile phase.
4. Wash the column with at least 20 CV of the binding mobile phase, or until the pH and/or conductivity at the outlet reach the corresponding values of the binding mobile phase.

Before analysis, it is recommended to run several blank runs without sample injection until the baseline is stable and reproducible.

7. Cleaning | Maintenance

Cleaning and maintenance of the column may improve its lifetime and increase reproducibility. Sample properties should be taken into account for column cleaning.

7.1. Cleaning in Place (CIP)

We recommend to clean the CIMac column after one day of analysis or after observing decreased column performance.

⚠**CAUTION**
Remain below the maximum pressure specified in Technical Data.

⚠**CAUTION**
Ensure compatibility between the current column solution and cleaning solutions (see examples in General Recommendations).

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1. If needed wash the column with 20 CV of water to prevent mixing of incompatible buffers.
2. Wash the column with at least 20 CV of 0.5 M NaOH. A contact time of up to 30 min is recommended.
3. Wash the column with 20 CV of water.
4. Wash the column with at least 20 CV of a neutralisation-equilibration solution. A buffer (e.g. Tris pH 7) with high salt concentration is recommended (e.g. binding mobile phase). A solution of 1 M ammonium acetate may be used. Note: Collect ammonium acetate solution in a separate waste container to avoid mixing with NaOH.

To improve cleaning, extend the contact time with cleaning solution or implement cleaning steps specific to the contaminants present in the sample.

8. Storage

Clean and equilibrate the column before storage. The column can be stored in working buffers overnight.

NOTICE
NaOH-ethanol mixtures at any concentration form ethoxide anions that are highly destructive to biomolecules, and ligands on chromatography media. Neutralise the column environment before introducing ethanol.

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1. Wash the column with 10 CV deionised water.
2. Wash the column with 10 CV of storage solution. **Note:** Reduce the flow rate when using viscous solvents (such as ethanol) to avoid a pressure increase.
3. Seal the column with blind fittings and store at the temperature specified in Technical Data. If there is a possibility of biological contamination from the sample it is recommended to store the column between 2 °C (36 °F) and 8 °C (46 °F).

9. Troubleshooting

Problems arising during the analysis are usually related to the column, sample, mobile phase, or the instrumentation. It is advisable to use an elimination approach to exclude possible causes. Please refer to our troubleshooting guide (biaseparations.com/en/library/guidelines).

10. Decommissioning | Transportation

If there is reason to return the product, complete a Return Form (biaseparations.com/en/terms-conditions) and contact help.bia@sartorius.com.

Contaminated samples used during the process that could cause biological or chemical hazards are potentially hazardous substances. If the product has come into contact with hazardous substances, steps must be taken to ensure proper decontamination and declaration.

Procedure
Decontaminate the product. The operator of the product is responsible for adhering to local government regulations on the proper decontamination and declaration for transport and disposal.

11. Ordering Information

Transferring the workflow to a different scale or format (analytical, screening) is simple with CIM®. Contact your local support to find the appropriate products.

Purification Scale Products

Catalog number	Product name
311.1219-2	CIMmultus® Oligo dT18 1 mL Monolithic Column (C12 Linker) (2 µm channels)
414.1219-2	CIMmultus® Oligo dT18 4 mL Monolithic Column (C12 Linker) (2 µm channels)
411.1219-2	CIMmultus® Oligo dT18 8 mL Monolithic Column (C12 Linker) (2 µm channels)
614.1219-2	CIMmultus® Oligo dT18 40 mL Monolithic Column (C12 Linker) (2 µm channels)
611.1219-2	CIMmultus® Oligo dT18 80 mL Monolithic Column (C12 Linker) (2 µm channels)
814.1219-2	CIMmultus® Oligo dT18 400 mL Monolithic Column (C12 Linker) (2 µm channels)
811.1219-2	CIMmultus® Oligo dT18 800 mL Monolithic Column (C12 Linker) (2 µm channels)
1014.1219-2	CIMmultus® Oligo dT18 4000 mL Monolithic Column (C12 Linker) (2 µm channels)
1011.1219-2	CIMmultus® Oligo dT18 8000 mL Monolithic Column (C12 Linker) (2 µm channels)

Screening Solutions

Catalog number	Product name
BIA-122.1219-2	CIM® Oligo dT18 0.05 mL Monolithic 96-well Plate (C12 Linker) (2 µm channels)
120.1219-2	CIM® Oligo dT18 0.2 mL Monolithic 96-well Plate (C12 Linker) (2 µm channels)

Sartorius BIA Separations d.o.o.
Mirce 21
SI-5270 Ajdovščina
Phone +386 59 699 500
www.biaseparations.com

The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote the other gender as well.

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