

Instructions for Use

CIMac PrimaS[®] 0.1 mL Analytical Column (2 µm channels)

CIM Convective Interaction Media[®]
110.5118-2



SARTORIUS

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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



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.

PrimaS® is a multimodal ligand which exploits a combination of anion exchange and hydrogen bonding to achieve unique selectivity. 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	Multimodal (anion exchange-hydrogen bonding)
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
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 40 °C (104 °F)
Chemical stability	All commonly used aqueous buffers, 0.1 M HCl, 500 mM acetic acid, 500 mM phosphoric acid, 2% benzyl alcohol, 0.1 M NaOH (tested up to 120 min), and 20 % ethanol solution. Avoid oxidizing agents. Avoid prolonged use of concentrated acids (more than 0.5 M) like hydrochloric or sulphuric acid. Avoid > 0.1 M NaOH solution.
Recommended pH	Working range 2–11, cleaning in place 1–13
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).
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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.

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.

6.3. Strip | Regeneration

A strip is typically implemented in the analytical run to remove tightly-bound sample components. It is common to use the same approach as the elution: elevated salt concentration (e.g. 2 M NaCl), change in pH (low pH or high pH solution), or other.

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)

In some cases, a simple regeneration of the monolithic column is insufficient. Sample molecules may not completely elute from the column or may even precipitate on the column. This build-up of contaminants on the monolithic column may cause loss of resolution and binding capacity, increased back pressure, or a complete blockage of the column. A specific CIP procedure should be considered for the type of contaminants present in the sample. An example of a general CIP procedure is presented below.

CAUTION

In case of pressure increase during cleaning, adjust flow rate to remain below the maximum pressure allowed over the column.

Perform the following procedure at up to half the maximum operating flow rate. This will ensure sufficient contact time between the monolith and cleaning solution. Optionally, if hydrophobic impurities are expected, wash with 10 CV of deionized water followed by 10 CV of 30 % 2-propanol.

1. Wash the column with at least 10 CV of deionised water.
2. Wash the column with at least 10 CV of a cleaning solution containing 0.1 M NaOH and 1 M NaCl. Note: Concentrations of NaOH higher than 0.1 M will irreversibly damage the column. See chemical stability under Characteristics of the monolith.
3. Wash the column with at least 10 CV of deionised water.
4. To reduce the pH, wash the column with at least 20 CV of a solution containing 0.1 M acetic acid and 1 M NaCl at pH 5. Other concentrated buffer (e.g. 0.1–0.5 M buffer, pH 5–6) can be used.
5. Wash the column with at least 10 CV of deionised water.

Note: NaOH forms a precipitate with bivalent metal cations (e.g. Mg^{2+} , Ca^{2+}). Precipitation causes a gradual pressure increase over consecutive runs until complete column blockage. The precipitate can be dissolved with a 0.1 M HCl wash. Consecutive washes with acid will have negative impact on column lifetime. To prevent precipitation, wash the column with at least 10 CV of water or a compatible buffer before and after NaOH.

8. Storage

The column can be stored in working buffers overnight. Before long term storage, clean and equilibrate the column.

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.

1. Wash the column with 10 CV of 0.1 M acetic acid at pH 5. Other concentrated buffer (e.g. 0.1–0.5 M buffer, pH 5–6) can be used.
2. Wash the column with 10 CV of deionised water.
3. 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.
4. 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.

Screening Solutions

Catalog number	Product name
BIA-122.5118-2	CIM PrimaS® 0.05 mL Monolithic 96-well Plate (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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