Instructions for Use

CIMac C4 HLD 0.1 mL Analytical Column (HLD Butyl) (2 µm channels)

CIM Convective Interaction Media®
110.8136-2

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	

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.

This high-performance analytical column is primarily intended for fast, efficient and reproducible analysis and in-process control of biomolecules such as pDNA, proteins and viral particles. It is used under hydrophobic conditions and can be implemented throughout different purification steps. The following information is being 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	C4 HLD (hydrophobic; butyl – high ligand density)
Colour code	White
Channel radius	1050 nm (950 nm – 1150 nm)
Support matrix	Poly(butyl 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	N.D.
Dynamic binding capacity	2.5–3.5 mg pEGFP/mL wet support, pEGFP (4.7 kbp) 0.04 mg/mL, 50 mM TRIS, 10 mM EDTA, 3 M $(NH_4)_2SO_4$, pH 7.2
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, such as 1 M NaOH, 0.1 M HCl, 3 M ammonium sulphate, 8 M urea and 6 M guanidine hydrochloride. Avoid oxidising agents and organic solvents, such as methanol, ethanol, acetonitrile, 2-propanol. Avoid prolonged use of concentrated acids (more than 0.5 M) such as hydrochloric, sulphuric or acetic acid.
Recommended pH	Working range 3–13, cleaning in place 1–14
Storage conditions	2 °C (36 °F) to 25 °C (77 °F); store in 10 mM NaOH.
Shelf life	7 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.

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 step with water can be used to regenerate the column. A regeneration step can be included into the operating method to improve reproducibility, to detect the resulting peak, or collect the eluted material.

1. Wash the column with at least 20 CV of low ionic strength mobile phase.
2. Re-equilibrate the column with at least 20 CV of binding mobile phase.

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 designed for the type of contaminants present in the sample. An example of a general CIP procedure is presented below.

Use a flow rate of 5 CV/min unless otherwise indicated and reverse the flow direction. This will ensure sufficient contact time between the monolith and cleaning solution and displace any particles accumulated at the inlet.

1. Wash the column with at least 20 CV of deionised water.
2. Wash the column with at least 20 CV of 1 M NaOH.
3. Wash the column with at least 20 CV of deionised water.
4. Wash the column with at least 20 CV of a concentrated buffer (e.g. 0.1–0.5 M buffer) to restore the appropriate pH.
Note: Binding buffer may be used for this step. Wash until the pH value at the outlet of the column corresponds to the buffer's pH.

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

Note: If CIP does not restore column performance completely, consider performing sanitisation of the column (see below for further instructions).

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
311.8136-2	CIMmultus® C4 HLD 1 mL Monolithic Column (HLD Butyl) (2 µm channels)
414.8136-2	CIMmultus® C4 HLD 4 mL Monolithic Column (HLD Butyl) (2 µm channels)
411.8136-2	CIMmultus® C4 HLD 8 mL Monolithic Column (HLD Butyl) (2 µm channels)
614.8136-2	CIMmultus® C4 HLD 40 mL Monolithic Column (HLD Butyl) (2 µm channels)
611.8136-2	CIMmultus® C4 HLD 80 mL Monolithic Column (HLD Butyl) (2 µm channels)
814.8136-2	CIMmultus® C4 HLD 400 mL Monolithic Column (HLD Butyl) (2 µm channels)
811.8136-2	CIMmultus® C4 HLD 800 mL Monolithic Column (HLD Butyl) (2 µm channels)
1014.8136-2	CIMmultus® C4 HLD 4000 mL Monolithic Column (HLD Butyl) (2 µm channels)
1011.8136-2	CIMmultus® C4 HLD 8000 mL Monolithic Column (HLD Butyl) (2 µm channels)

Screening Solutions

Catalog number	Product name
BIA-122.8136-2	CIM® C4 HLD 0.05 mL Monolithic 96-well Plate (2 µm channels)
120.8136-2	CIM® C4 HLD 0.2 mL Monolithic 96-well Plate (2 µm channels)

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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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