Instructions for Use

CIMac r-Protein L 0.1 mL Analytical Column (Recombinant Protein L) (1.3 µm channels)

CIM Convective Interaction Media®
110.1021-1.3

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.

CIMac r-Protein L Analytical Columns are primarily intended for fast, efficient, and reproducible analysis of a wide range of antibodies and antibody fragments such as Fabs, single-chain variable fragments (scFv) and domain antibodies (Dabs). They can be used for quantitative and qualitative in-process and final control. 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	r-Protein L (affinity; recombinant protein L produced from E. Coli coupled to CDI-preactivated support)
Colour code	White and red
Channel radius	675 nm (600 nm – 750 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	N.D.
Dynamic binding capacity	N.D.
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, 8 M urea, 6 M guanidine hydrochloride and 20 % ethanol solution.
Recommended pH	Working range 2–10, cleaning in place 2–12.5
Storage conditions	2 °C (36 °F) to 8 °C (46 °F); 20 % EtOH in 20 mM TRIS pH 7.4
Shelf life	2 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). Wash the column with water or another compatible solution when using two incompatible solutions consecutively.

5.2. Buffer Selection

The most commonly used bind/wash buffers are sodium phosphate buffer and TRIS buffer (25–100 mM; 50 mM is recommended as starting concentration). Other buffers with pH between 6 and 9 can be used (pH 7.4 is recommended as a starting point). Device is compatible with neutral salts (e.g. sodium chloride or potassium chloride). Salt concentrations of 0.1–0.2 M can improve the peak shape and/or solute recovery.
Elution should be performed under low pH conditions using a suitable buffer. Elution in pH ranging from 2 to 3.5 results in high yield. The most commonly used buffers are citric acid and acetic acid in pH range 2.3–3.5 and in concentrations from 0.05–0.5 M.

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

Regeneration of the column: removal of ionically bound compounds from the monolithic column, followed by column re-equilibration:

1. Flush the column with at least 20 CV of 0.1 M buffer containing 1.0 M NaCl, pH 7–8 at the operating flow rate.
2. Flush the column with 20 CV of concentrated elution buffer (e.g. 0.1 M glycine, pH 2.0, or 0.5 M acetic acid, pH 2.5).
3. Re-equilibrate the column with at least 20 CV of binding mobile phase.

Regeneration of the column: removal of hydrophobically bound compounds from the monolithic column, followed by column re-equilibration:

1. Flush the column with at least 20 CV of 0.1 M buffer, pH 7–8 at the operating flow rate.
2. Flush the column with 20 CV solution of ethanol and buffer (20 mM TRIS, pH 7.4) in the ratio 1:4 (v/v).
3. Re-equilibrate the column with at least 20 CV of binding mobile phase.

To maintain the optimal efficiency of the column, it is advisable to perform the regeneration after each sequence of injections of samples.

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 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. In most cases, the following procedure can be used.

Use a flow rate of 2–5 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 10–20 CV of a cleaning solution containing of 15 mM NaOH and 1 M NaCl. **Note**: Do not exceed 30 minutes of contact time with the cleaning solution in a single cleaning cycle.
2. Wash the column with at least 20 CV of deionised water.
3. 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.
4. Equilibrate the column by following the ‘Equilibration’ procedure.

⚠CAUTION
Adjust the flow rate when washing a column with cleaning solutions to prevent an increase in back-pressure. Do not exceed the maximum pressure allowed over the column.

Note: Higher concentration of NaOH (for example 0.1 M) can be exceptionally used for CIP, but consecutive application of such CIP procedure results in deterioration of the column.
Note: The CIP solution in the procedure above can be replaced by 8 M urea in acidic buffer (e.g. 100 mM acetic acid, pH 2.5).

8. 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).

9. 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.

10. 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.

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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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