Instructions for Use

CIMmultus PrimaS® 800 mL Monolithic Column (2 µm channels)

CIM Convective Interaction Media®
811.5118-2

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.

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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

CIMmultus® Monoliths are reusable chromatography devices for scalable high-resolution purification of complex biological samples. Inside the custom designed housing is a single-piece stationary phase with homogeneous channel size and surface chemistry. Without the need for column packing, CIMmultus® Monoliths are ready for use out of the box.

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	Outer diameter: 105 mm; inner diameter: 65 mm; length: 150 mm; bed volume (CV): 800 mL
Connector	TC 1 in. (25 mm), 8 mm ID bore
Operating flow rates	Up to 2 CV/min | 1600 mL/min | 245 cm/h. Do not go below 0.1 CV/min
Maximum pressure	1.4 MPa, 14 bar, 200 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

The linear flow rate can be calculated with the following equation and supporting data, which is available in the Technical Data.

$$Average\ linear\ velocity, u_{av} = \frac{F}{\pi \times L} \frac{ln\left(\frac{D_o}{D_i}\right)}{(D_o - D_i)}$$

F is the flow rate in mL/min, Do and Di are the outer and inner diameter of the column and L is the column length.

4. Device Overview | Description

The housing of this CIMmultus® column is made of epoxy thermoset material. Its surface is coated pinhole-free with biocompatible (USP Class VI) Parylene C.

NOTICE
Do not expose the column housing to pure acetone.

5. 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
Larger columns are shipped in a wooden crate, and a suitable stand is provided in the packaging. The columns have either a stand (400 | 800 mL columns) or wheels (4 | 8 | 40 L columns). Place them in an upright position on a flat surface. The 40 L column should be lifted from its crate by attaching straps to the lifting eye bolts on the housing.

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

6. Getting Started

Set the pressure relief valve to the maximum pressure allowed on the CIM column as indicated in Technical Data. Before using the column, an integrity test must be performed. Guideline 'Column integrity test' (biaseparations.com/en/library/guidelines) should be followed. It is advised to repeat this procedure regularly or when deviations in performance are observed.

NOTICE
The column should be equilibrated to working temperature for optimal results. Allow at least 12 h for the column to reach working temperature.

6.1. 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.

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7. Operating the Column

7.1. Connecting the Column

Position the column with the inlet at the bottom and outlet at the top by placing the 400 | 800 mL column on its stand. The 4000 | 8000 mL columns should stand upright on its wheels. Connect the column to the system with flow turned off in the following order:

1. Carefully remove the blind fitting on the inlet side and connect the inlet tubing.
2. Carefully remove the blind fitting on the outlet side and connect the outlet tubing.

Disconnect by reversing the steps above.

NOTICE
Do not open both inlet and oulet simultaneoulsy to avoid leaking of mobile phase. Changing the order of the above procedure might cause leakage of the mobile phase from the column and affect its performance!

NOTICE
Reversing the flow direction will damage the column. Make sure the column is connected according to the flow direction indicated by the arrow. The 40 L housing has an integrated non-return valve at the column outlet to prevent reversing the flow direction. Do not remove or disassemble the valve. **Note**: Software specific settings which regulate the flow direction should be checked. Ensure the correct flow mode is selected so that flow can go only in the direction indicated on the monolith.

NOTICE
Spikes in pressure generated during sudden pump fluctuations (e.g. immediate application of maximum flow rate or sudden pump stop at high operating pressure) can generate a backpressure shock, which can damage the monolith.

7.2. Equilibration

The column should be equilibrated with a suitable counter-ion. Binding buffer should have the same or similar composition to the loaded sample. To speed up equilibration, a buffer containing a higher concentration of the appropriate ion may be used (e.g. the elution buffer), as described here.

1. If needed wash the column with 10 CV of water to prevent mixing of incompatible buffers.
2. Wash the column with at least 10 CV of elution mobile phase (which contains elevated salt concentration). For weak ion exchangers, an extended contact time is recommended (by reducing the flow rate to < 0.5 CV/min or a hold step after the flush).
3. Wash the column with at least 10 CV of binding mobile phase. The composition of this mobile phase should be similar to the sample composition.

Use system detectors as indication of successful equilibration. Conductivity and pH at the outlet should match buffer specifications.

7.3. Strip | Regeneration

A strip is typically implemented in the purification 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.

8. 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.

8.1. 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. Mg2+, Ca2+). 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.

8.2. Sanitisation

Pump at least 10 CV of a cleaning solution (167 mM acetic acid, 120 mM phosphoric acid, and 2 % benzyl alcohol) through the column at up to half the maximum operating flow rate. Stop the pump and leave the column in contact with the cleaning solution for at least 2 h (up to 12 h if necessary) at room temperature. If needed, the column can be disconnected from the system and closed with blind fittings. Re-connect the column and proceed to 'Equilibration' Procedure.
Cleaning validation remains end user responsibility.

NOTICE
Ensure that the chromatography system and auxiliary components are compatible with the cleaning solution at the concentrations used.

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9. 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.

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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).

10. 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).

11. 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.

12. 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 cGMP Compliant

Catalog number	Product name
901.5118-2	CIMmultus PrimaS® 8 mL cGMP Compliant Monolithic Column (2 µm channels)
914.5118-2	CIMmultus PrimaS® 40 mL cGMP Compliant Monolithic Column (2 µm channels)
924.5118-2	CIMmultus PrimaS® 400 mL cGMP Compliant Monolithic Column (2 µm channels)
921.5118-2	CIMmultus PrimaS® 800 mL cGMP Compliant Monolithic Column (2 µm channels)
934.5118-2	CIMmultus PrimaS® 4000 mL cGMP Compliant Monolithic Column (2 µm channels)

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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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