

Instructions for Use

CIMmultus™ QA HR 4000 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)

CIM Convective Interaction Media®
BIA-934.5213-2



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

Column integrity test



Specimen QA HR Method Guide



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 after sanitisation and integrity test.

This high-performance ion exchange column is primarily intended for fast and efficient purification of biomolecules

such as large proteins, viruses and virus like particles through their ionic interaction with the monolith. QA HR is designed for even higher reproducibility for the most sensitive separations (e. g. enrichment of full AAV capsids). The QA HR release criteria are therefore based on empty AAV capsid elution using Specimen.

CIMmultus QA HR cGMP column (parental column) is accompanied with its Specimen QA HR delivered in the same shipping box. Specimen QA HR is a small non-GMP unit, extracted from the same batch of bulk monolith as its parental column. Specimen QA HR matches chromatographic properties of parental column, therefore Specimen units can be used to control lot-to-lot variabilities of their parental columns. Follow a method guide for Specimen QA HR (<https://www.biaseparations.com/en/library/brochures>).

NOTICE

Specimen should be treated in the same way as its parental column to represent chromatographic properties of parental column. Special attention should be paid to NaOH exposure (NaOH concentration and exposure time to NaOH solution) during sanitization and cleaning.

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	QA (strong anion exchanger; quaternary amine)
Channel radius	1050 nm (950 nm - 1150 nm)
Support matrix	Poly(glycidyl methacrylate -co- ethylene dimethacrylate)
Monolith dimensions	Outer diameter: 300 mm; inner diameter: 255 mm; length: 205 mm; bed volume (CV): 4000 mL
Connector	TC 1 in. (25 mm), 8 mm ID bore
Operating flow rates	Up to 1 CV/min 4000 mL/min 135 cm/h. Do not go below 0.1 CV/min
Maximum pressure	0.7 MPa, 7 bar, 100 psi
Operating temperature	4 °C (39 °F) to 40 °C (104 °F)
Chemical stability	All commonly used aqueous buffers, 0.1 M NaOH, 0.1 M HCl, 8 M urea, 6 M guanidine hydrochloride and 20 % ethanol solution. Avoid oxidizing agents. Avoid prolonged use of concentrated acids (more than 0.1 M) like hydrochloric, sulphuric or acetic acid. Avoid prolonged exposure in NaOH and unnecessary exposure in more than 0.1 M NaOH solutions.
Recommended pH	Working range 2–13, cleaning in place 1–13.7
Storage conditions	2 °C (36 °F) to 25 °C (77 °F); 20 % EtOH in 20 mM sodium acetate pH 5.5
Shelf life	N.D.

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

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

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 L columns). Place them in an upright position on a flat surface.

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 Characteristics of the monolith. 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.

NOTICE

This cGMP compliant column should be sanitised before first use. Follow the sanitisation protocol in this document (see chapter 8.2.) once the 'Column integrity test' has been completed.

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).
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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.

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 outlet simultaneously 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. **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).
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)

Column cleaning is recommended between purification runs or cycles. A reduced flow rate is suggested for column

cleaning to extend contact time with the cleaning and neutralisation-equilibration solutions.

⚠ 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).

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 cleaning solution with 0.1 M NaOH and 2 M NaCl (combined). Make sure the contact time is properly optimised. Take into account that amino groups like QA are not stable in NaOH solutions.
 3. If needed wash the column with 10 CV of water to prevent mixing of incompatible buffers.
 4. Wash the column with at least 10 CV of a neutralisation solution. 0.5 M phosphate buffer pH 6.5 or 1 M sodium acetate pH 5.5 are recommended to efficiently displace the counter ion. A solution of 1 M ammonium acetate can also be used. **Note:** Collect ammonium acetate solution in a separate waste container to avoid mixing with NaOH.
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⚠ CAUTION

Prolonged exposure to 0.1 M NaOH might affect column performance. Immediately wash the column with neutralisation solution as per instruction. Any modification of recommended cleaning protocol could result in shorter lifespan of the column.

8.2. Sanitisation

The procedure described here uses a standard cleaning solution with an extended contact time. A reduced flow rate is suggested to extend contact time with the cleaning and neutralisation-equilibration solutions.

⚠ CAUTION

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

1. Wash the column with 10 CV of water.
 2. Wash the column with at least 10 CV of sanitisation solution with 0.5 M NaOH and 2 M NaCl (combined).
 3. Stop the flow and leave the column in contact with the sanitisation solution for at least 1 h. Make sure the contact time is properly optimised. Take into account that amino groups like QA are not stable in NaOH solutions.
 4. If needed wash the column with 10 CV of water to prevent mixing of incompatible buffers.
 5. Wash the column with at least 10 CV of a neutralisation solution. 0.5 M phosphate buffer pH 6.5 or 1 M sodium acetate pH 5.5 are recommended to efficiently displace the counter ion. A solution of 1 M ammonium acetate can also be used. **Note:** Collect ammonium acetate solution in a separate waste container to avoid mixing with NaOH.
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⚠ CAUTION

Exposure to 0.5 M NaOH can affect column performance. Immediately wash the column with neutralisation solution as per instructions. Any modification of recommended cleaning protocol could result in shorter lifespan of the

column. Test the column performance after the sanitization to determine if it still fits your internal qualification parameters.

9. Storage

Before storage, follow instructions in Cleaning in Place section that will guide you through cleaning and neutralisation protocol.

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 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. **Note:** Any deviation from the recommended storage solution (i.e. 20% EtOH in 20 mM sodium acetate pH 5.5) could affect column performance.
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).

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@biaseparations.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
BIA-914.5213-2	CIMmultus™ QA HR 40 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-911.5213-2	CIMmultus™ QA HR 80 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-924.5213-2	CIMmultus™ QA HR 400 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-921.5213-2	CIMmultus™ QA HR 800 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-934.5213-2	CIMmultus™ QA HR 4000 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-931.5213-2	CIMmultus™ QA HR 8000 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)

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The information and figures contained in these instructions correspond to the version date specified below.

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