Instructions for Use

CIMmultus® r-Protein G 8 mL Monolithic Column (Recombinant Protein G) (1.3 µm channels)

CIM Convective Interaction Media®
411.1011-1.3

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	

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. This stationary phase is designed to simplify method transfer from development into production. Without the need for column packing, CIMmultus® Monoliths are ready for use out of the box.

r-Protein G columns are used for fast and efficient purification of antibodies from different samples. The antibody binds to the immobilised protein through the Fc portion, leaving the antigen binding site free. The range of polyclonal IgGs that bind strongly to protein G is greater than on protein A and it works on a wider pH range. 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 G (affinity, recombinant protein G produced from E. Coli coupled to CDI-preactivated support)
Channel radius	675 nm (600 nm – 750 nm)
Support matrix	Poly(glycidyl methacrylate -co- ethylene dimethacrylate)
Monolith dimensions	Outer diameter: 15 mm; inner diameter: 6.5 mm; length: 56 mm; bed volume (CV): 8 mL
Connector	5/16-24 UNF coned port, 1/8'' OD tubing connection (Manufactured before Oct 2019, Adapter 422.0900 included) or 10-32 UNF coned port, 1/16'' OD tubing connection (Manufactured from Oct 2019)
Ligand density	N.D.
Dynamic binding capacity	≥ 10 mg hIgG/mL wet support, human IgG 1.0 mg/mL, 20 mM Tris-HCl buffer, pH 7.4, flow rate 16 mL/min, Human IgG: octagam® 5% (Octapharma®)
Operating flow rates	Up to 12.5 CV/min | 100 mL/min | 335 cm/h. Do not go below 0.1 CV/min
Maximum pressure	2.0 MPa, 20 bar, 290 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 2–11, cleaning in place 2–13
Storage conditions	2 °C (36 °F) to 8 °C (46 °F); 20 % EtOH in 20 mM Tris pH 7.4
Shelf life	2 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). Wash the column with water or another compatible solution when using two incompatible solutions consecutively.

6.2. Buffer Selection

The most commonly used bind/wash buffers are sodium phosphate buffer and TRIS buffer (25 to 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 to 0.2 M can improve the peak shape and/or solute recovery.

Elution should be performed under low pH conditions using a suitable buffer. The most commonly used buffers are citric acid, glycine and acetic acid. The table below summarizes these buffers, their concentration and pH range.

Table 2: Elution buffers and their pH range.

Buffer	Concentration	pH range
Glycine	0.1 M	2.0–3.0
Citric acid	0.1 M	2.5–3.0
Acetic acid	5–20%	2.5–3.0

7. Operating the Column

7.1. Connecting the Column

Column should be connected according to the flow direction indicated by the arrow and with the inlet placed at the bottom. 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. 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 before use. Equilibration mobile phase should be of same or similar composition to the sample.

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.
3. Wash the column with at least 10 CV of binding mobile phase.

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

7.3. 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.

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 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 procedure below can be used.

⚠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.
Removal of precipitated proteins:

1. Wash the column with at least 5 CV of a cleaning solution containing 0.1 M NaOH and 1 M NaCl. **Note:** Do not exceed 10 minutes of contact time with the cleaning solution in a single cleaning cycle.
2. Wash the column with at least 10 CV of deionised water.
3. Wash the column with at least 10 CV of a concentrated buffer (e.g. 0.1–0.5 M buffer) to neutralize the column to the appropriate pH.

Removal of strongly bound hydrophobic proteins or lipids:

1. Wash the column with at least 10 CV of deionised water.
2. Wash the column with at least 10 CV of 30% 2-propanol.
3. Wash the column with at least 10 CV of deionized water.

Note: Up to 0.5 M NaOH and 1 M NaCl could be exceptionally used for CIP procedure, but consecutive application of such CIP procedure results in deterioration of column performance.

9. 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).

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 non-cGMP Compliant

Catalog number	Product name
311.1011-1.3	CIMmultus® r-Protein G 1 mL Monolithic Column (Recombinant Protein G) (1.3 µm channels)
411.1011-1.3	CIMmultus® r-Protein G 8 mL Monolithic Column (Recombinant Protein G) (1.3 µm channels)
411.1011-1.3	CIMmultus® r-Protein G 8 mL Monolithic Column (Recombinant Protein G) (1.3 µm channels)
611.1011-1.3	CIMmultus® r-Protein G 80 mL Monolithic Column (Recombinant Protein G) (1.3 µm channels)
611.1011-1.3	CIMmultus® r-Protein G 80 mL Monolithic Column (Recombinant Protein G) (1.3 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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