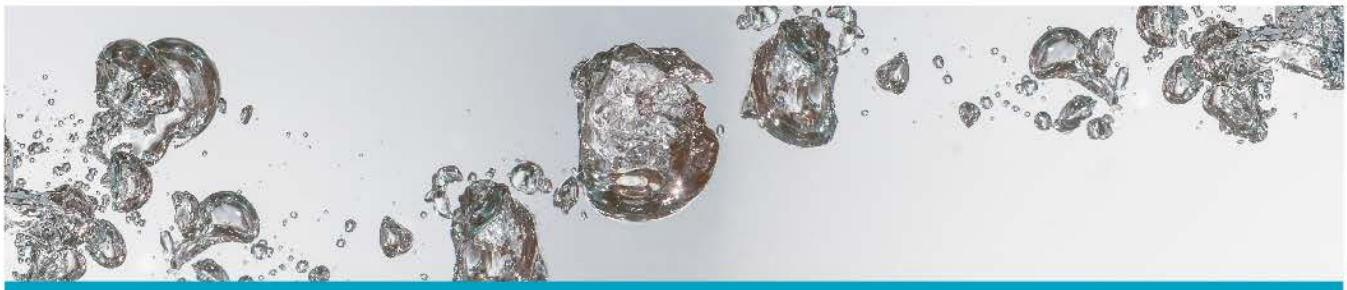

CIMmultus™ 8 mL Advanced Composite Column

ABOUT CIMmultus™ Advanced Composite Columns

CIMmultus™ is the 'multi-use disposable' product line (Figure 1), available in non-cGMP and cGMP compliant version. A CIMmultus™ multi-use Advanced Composite Column is an ideal choice for efficient laboratory and large-scale purification of biopharmaceuticals in the area of viral and non-viral vaccine and vector production, therapeutic proteins and antibody purification.

CIMmultus™ housing is made of epoxy thermoset resin coated with biocompatible (USP Class VI) Parylene C and was developed according to the Sound Engineering Practice guidelines. The column design directs the mobile phase from the outer to the inner surface of the monolith (radial flow, see Figure 2). With radial flow, high resolution is preserved during scale-up while maintaining a short separation path length. The thin monolithic layer and the specially engineered highly porous structure allow chromatographic separations at high flow rates.

Figure 1: CIMmultus™ Columns

This monolithic column is easy to use. The advantageous features of CIMmultus™ Advanced Composite Column include:

- Use with any HPLC, LC, peristaltic pump or syringe with appropriate adapters
- Fast large scale separations of biomolecules
- Flow-independent resolution
- Flow-independent dynamic binding capacity
- High resolution at increased sample loading
- Low back pressure at high flow rates
- Fast column equilibration
- pH stability over a wide range

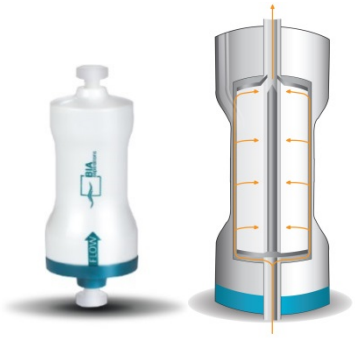

Figure 2: CIMmultus™ 8 mL Advanced Composite Column (left) operates with unique 'radial flow' direction (right) whereby solutes enter the stationary phase across the length of the tubular monolith; separation takes place across the width of the tube wall.

CIMmultus™ Advanced Composite Columns are available in a number of chemistries (i.e. QA, DEAE, SO3, C4 HLD, and OH). The catalogue number, chemistry and nominal volume of the monolith, batch number, expiry date and maximum operating pressure are marked on the label. Each column is individually tested and is delivered with a Certificate of Analysis.

For more detailed information about material characteristics, please refer to Product Specific Information Sheet (PSIS) for individual chemistry of the monolith.

Publication #: IM-M8-1604

UNPACKING

Carefully inspect the product for any damage that may have occurred during shipping. Immediately report any such damage to your vendor and the courier.

CIMmultus™ Advanced Composite Column is shipped at room temperature in a suitable storage solution and should be stored between 2 °C (36 °F) and 25 °C (77 °F) until use.

WARNING: Do not store the CIMmultus™ Advanced Composite Column at ≤ 0 °C (32 °F).

BEFORE YOU BEGIN

Use the product according to the guidelines in this Instruction Manual. Improper use may result in malfunction, personal injury, or damage of the product or material. Follow general safety instructions for laboratory work.

PREPARATION OF CIMmultus™ 8 mL Advanced Composite Column FOR USE

1. Set the pressure relief valve of the system (pump) to the value which is smaller or equal (≤ X+Y) to the sum of the system pressure (measured without column connected to the system = X bars) and maximum allowed pressure over the column (=Y bars). For maximum allowed pressure over the column refer to the PSIS.
2. Perform the test procedure according to the Guideline ´Column performance test` (www.biaseparations.com). It is mandatory to test the CIMmultus™ Advanced Composite Column before the first use and it is recommended to verify the column integrity with the same procedure regularly.

The CIMmultus™ Advanced Composite Column should be connected to the system according to the following steps:

- Place the column in a horizontal position.
- Carefully remove the blind fitting on the inlet side and connect the inlet tubing to the connector.
- Carefully remove the blind fitting on the outlet side and connect the column to the HPLC/LC system with the outlet tubing using appropriate connectors (please refer to the PSIS).

WARNING: Do not change the order of the above procedure since it might cause leakage of the mobile phase from the column and consequently affect column performance!

Equilibrating CIMmultus™ Advanced Composite Column

The column should be equilibrated according to the following procedure:

1. Wash the CIMmultus™ Advanced Composite Column with at least 40 mL (5 column volumes; CV) of deionized water, followed by 40 mL (5 CV) of binding mobile phase (e.g. for ion-exchange chromatography a low ionic strength buffer) at low flow rate (2 CV/min; 16 mL/min). **Note:** Allow 2 CV (16 mL) of deionized water to flow into a waste container without going through the detector cell. This will remove any small particles or air bubbles that may affect the detector cell.
2. Wash the CIMmultus™ Advanced Composite Column with at least 40 mL (5 CV) of the eluting mobile phase (e.g. for ion-exchange chromatography a buffer containing high concentration of salt).
3. Wash the CIMmultus™ Advanced Composite Column with at least 80 mL (10 CV) of the binding mobile phase or until pH and/or conductivity at the outlet reaches corresponding values of the binding mobile phase.
4. The CIMmultus™ Advanced Composite Column is now ready for use. For details on operating conditions refer to the PSIS.

ENSURING OPTIMAL PERFORMANCE OF CIMmultus™ Advanced Composite Column

General recommendations

To extend the lifetime of your CIMmultus™ Advanced Composite Column, follow these guidelines:

- Mobile phases (buffers) should be freshly prepared and filtered through at least a 0.45 µm filter.
- 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' (www.biaseparations.com).
- Do not disassemble the CIMmultus™ Advanced Composite Column.
- Do not let the CIMmultus™ Advanced Composite Column dry out.

Regeneration Procedure

Regeneration of the column starts with the removal of bound substances from the stationary phase, followed by re-equilibration with the appropriate counter-ion. The following method is recommended:

Wash the column with at least 160 mL (20 CV) of buffer containing 2 M NaCl (for CIMmultus QA, DEAE and SO3 chemistries) or buffer containing no salt (for CIMmultus C4 HLD and OH chemistries) at the operating flow rate (for details on operating conditions refer to the PSIS). Equilibrate the column according to the procedure described in the section 'Equilibrating CIMmultus™ Advanced Composite Column' above.

Publication #: IM-M8-1604

Cleaning In Place (CIP) Procedure

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 protocol should be designed according to the type of contaminants present in the sample. An example of a general CIP procedure is presented below:

1. Wash the CIMmultus™ Advanced Composite Column with 80 to 160 mL (10 to 20 CV) of the cleaning solution containing 1 M NaOH and 2 M NaCl. **Note:** Use a low flow rate (8 mL/min; 1 CV/min) to ensure exposure of the column to the cleaning solution for several minutes.
2. Wash the column with 80 to 160 mL (10 to 20 CV) of deionized water.
3. Wash the column with 80 to 160 mL (10 to 20 CV) of a concentrated buffer (e.g. 0.1 M to 0.5 M buffer) to restore the appropriate pH.
4. Re-equilibrate the column with at least 160 mL (20 CV) of the binding mobile phase (buffer).

Sanitization Procedure

CIMmultus™ Advanced Composite Column can be sanitized. Pump 80 to 160 mL (10 to 20 CV) of a cleaning solution (1 M NaOH and 2 M NaCl) through the column at low flow rate (8 mL/min; 1 CV/min). Stop the pump and leave the column in contact with the cleaning solution for at least 2 h (up to 12 h) at room temperature. If needed the column can be disconnected from the system, sealed with blind fittings and after sanitization re-connected to the system. After sanitization wash the column following steps 2. to 4. of the general CIP procedure described above.

WARNING: Ensure that the chromatography system and auxiliary components are compatible with NaOH at the concentrations used. Follow all safety regulations when handling with NaOH solutions.

Storage

Before the storage the column should be washed with 80 mL (10 CV) of low salt buffer followed by at least 80 mL (10 CV) of deionized water and flushed with at least 160 mL (20 CV) of storage solution (i.e. 20 % ethanol for DEAE, QA, SO3 and OH chemistry or 10 mM NaOH for C4 HLD chemistry) at an operating flow rate. The column should be sealed with blind fittings and stored at 2 °C (36 °F) to 25 °C (77 °F). If there is a possibility of biological contamination from the sample it is recommended that the column is stored at 2 (36 °F) to 8 °C (46 °F). The final decision on storage T should be determined by the user.

RETURN OF THE COLUMN

The returned Column should be accompanied with completed Return Form (www.biaseparations.com).

TROUBLESHOOTING

Problem	Possible source	Action
Increased back pressure	Blockage in monolithic column, mixing tee, capillaries, in-line high pressure filter, or detector cell.	• Exchange capillary, in-line filter, or mixing tee. • Clean the detector cell. • Filter mobile phases. • Perform regeneration and/or CIP procedure of the CIMmultus™ Advanced Composite Column.
Poor resolution (i. e. wider peaks)	Various	• Perform regeneration and/or CIP procedure
Poor or no baseline separation	Various	• Make shallower gradient. • Check the pH and the composition of the mobile phase. • Verify that the column chemistry is appropriate for your application.

For any additional information or further enquiries please contact us:

Tel.: +386 59 699 500

tech-support@monoliths.com
sales@biaseparations.com

www.biaseparations.com

Publication #: IM-M8-1604