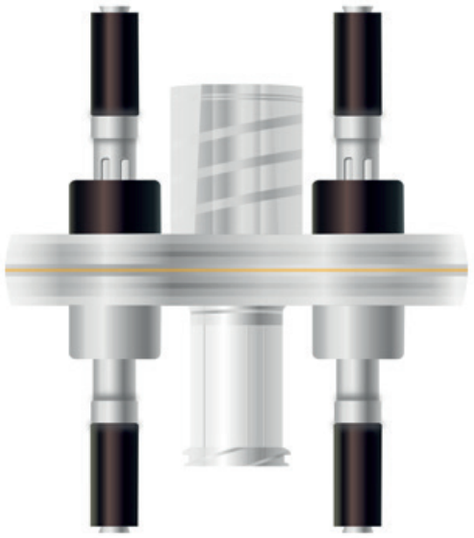
Instruction for use

i3 DMC^{CEX}

CEX Membrane Adsorber



REF DMC301

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1 Intended use

The i3 DMC^{CEX} is intended for the purification of positively charged biomolecules using electric potential for elution. It enables a rapid purification of highly concentrated eluates for drug discovery applications and in the production of biomolecules for research and development purposes. the i3 DMC^{CEX} Membrane Adsorber can be operated with a peristaltic pump or chromatography system.

2 Application

- Ideal for rapid capture and release of highly concentrated positively charged biomolecules (e.g. proteins).
- Enables working in small scale applications, such as research and process development.
- Ideal for sample preparation in low buffer media e.g. mass spectrometry

3 Safety instructions

1. Do not use the filter outside of its specifications.
2. No organic solvents should be used.
3. Do not exceed the maximum pressure.
4. Do not touch the inlet and outlet electrical pins during DMC elution operation.
5. Do not sterilize or sanitize.

4 Safety symbols

Article number

Non steril product

Do not use if the device or the packaging is damaged

Read the instructions thoroughly before use

LOT number

Manufacture

5 Technical data

Membrane material:	Polyamide (PA)
Pore size:	0.2 µm
Effective filtration area:	Ø 22.0 mm | 0.89"
Membrane thickness:	0.11 mm | 0.004"
Bed volume:	0.04 mL
Dynamic binding capacity per unit*:	up to 1.2 mg Lysozyme
Recommended operating flow rate:	2.0 mL/min
Maximum operating pressure:	3 bar | 43.5 psi
Recommended elution voltage:	+2.0 V
Temperature stability:	4° C-RT | 39.2° F-RT
Connectors:	Luer-Lock
Autoclavability:	Non autoclavable
Dimensions:	H: 34.0 mm | 1.3"
	Ø: 29.7 mm | 1.2"

* Determined in 0.24 mM PBS, pH 7.4

** i3 standard PBS working solution: 0.24 mM phosphate buffer, 2.74 mM NaCl and 0.054 mM KCl

6 Installation

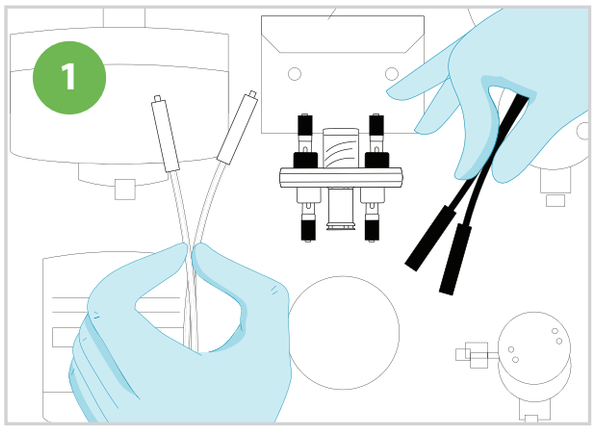
To install the i3 DMC^{CEX} Membrane Adsorber, you will need a liquid chromatography system (LC) with a Luer-Lock connector. Additionally, when utilizing the DMC elution technique, it is essential to have the i3 DMC$_{Control}$ Smart Control Unit with uniquely designed connecting cables.

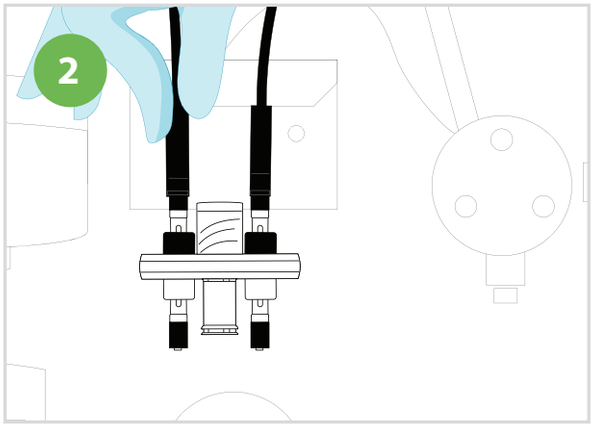
6.1 Installation of i3 DMC^{CEX}

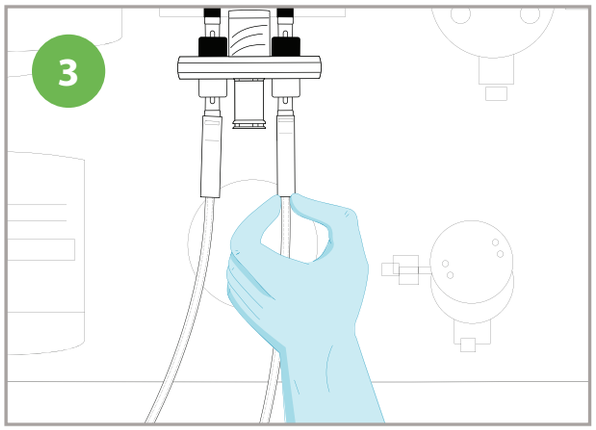
Follow the detailed step-by-step assembly instructions for installation of i3 DMC^{CEX}. Ensure that the membrane adsorber is securely connected to the i3 DMC$_{Control}$ and LC system. Loose connections may lead to suboptimal performance or device malfunction.

6.1.1 Installation to i3 DMC$_{Control}$

Utilizing the DMC elution method, it is essential to have the i3 DMC$_{Control}$ with uniquely designed connecting cables. Following steps describe the connection of i3 DMC$_{Control}$ and appropriate cables for i3 DMC^{CEX}. Place the device near the Membrane Adsorber to facilitate efficient operation. Avoid excessive distances between the device and the Membrane Adsorber and ensure that the contact cables are not under tension. Place the device in a location where the cables can naturally and freely connect to the inlet and outlet pins of the Membrane Adsorber without unnecessary strain. Turn on the device and connect the cables before initiating the self-check. Identify the appropriate cables for connection (picture 1). Refer to the user manual to ensure the correct cables are selected. Contact the corresponding cables with the inlet and outlet pins of the Membrane Adsorber (picture 2+3). To perform an experiment with the Membrane Adsorber and i3 DMC$_{Control}$ follow the user manual of i3 DMC$_{Control}$.

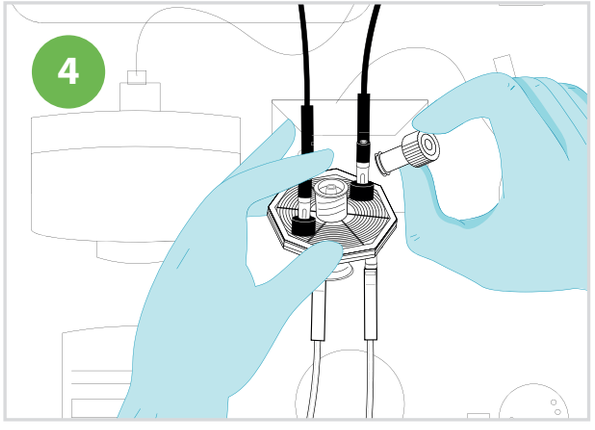


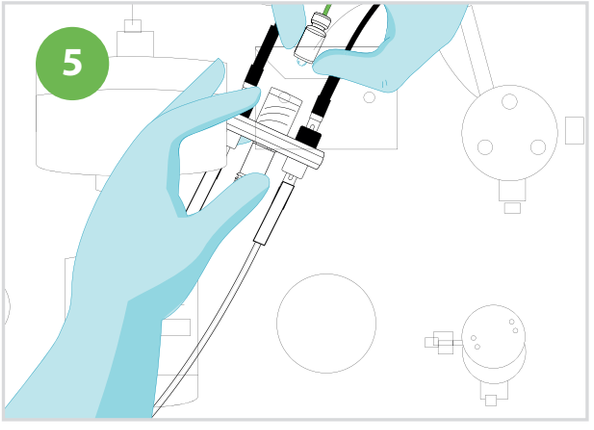


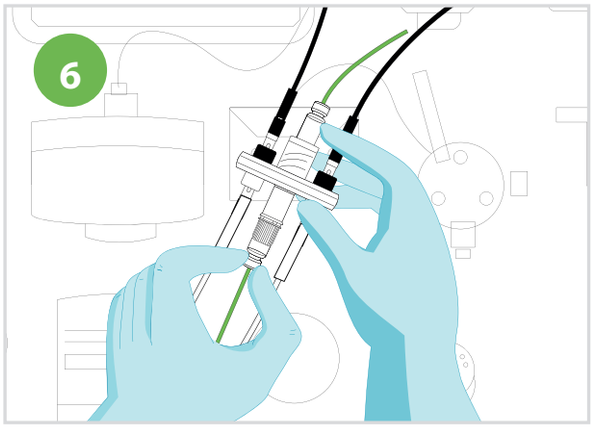


6.1.2 Installation to liquid chromatography (LC) systems

For the operation of the Membrane Adsorber with LC system, first remove both caps of the Membrane Adsorber and start your fluid flow (picture 4). Once fluid begins to emerge, connect the tubing to the inlet of the prefilled Membrane Adsorber using a Luer-Lock adapter (picture 5). Ensure that no air is introduced into the system. Next, turn the filter against the flow and wait until the fluid comes out on the other side. If air is introduced into the Membrane Adsorber, gently tap the unit to center air bubbles. If no air bubbles are present, turn the filter back and connect the tubing to chromatography system (picture 6). Use a tissue to dry the unit after filling process. In case larger air bubbles have entered the unit, follow the steps outlined in section 6.1.3.





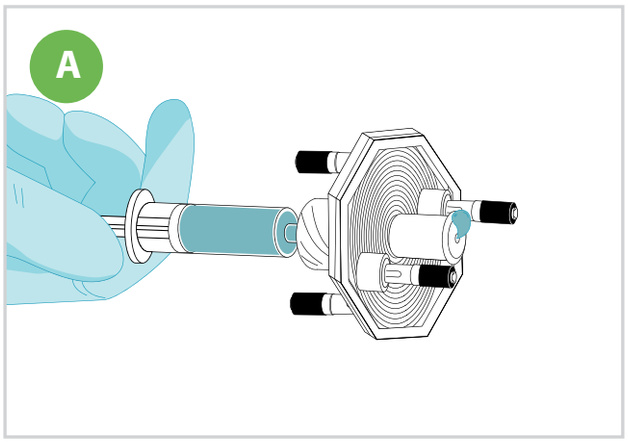


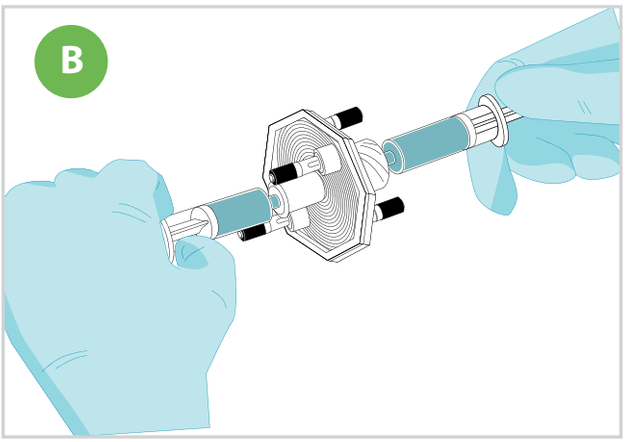
6.1.3 Venting

During the venting process of the Membrane Adsorber, do not exceed a pressure of 3 bar (0.3 MPa | 43.5 psi), as excessive pressure may damage the membrane or the filter housing.

To ensure proper venting, fill two 5 mL Luer syringes with the operating buffer. Ensure that no air is trapped in the syringe. Connect one syringe to the female Luer Lock of the Membrane Adsorber. Gradually fill the upper part of the unit until a drop appears at the bottom of the syringe filter (see picture A).
Next, attach the second syringe to the male Luer-Lock at the bottom and fill the lower part with the operating buffer (see picture B).

If air is still present in the filled unit, remove the bottom syringe and reposition the air bubbles in the centre of the upper section of the unit.
Reconnect the syringe to the bottom part of the unit and gently backflush to expel the air from the upper part.





7 Operation

To ensure efficient purification using i3 DMC^{CEX} Membrane Adsorber with DMC elution method, follow the procedures outlined in the subsequent sections. Specific procedures will need to be developed on a case-by-case basis. Optimal conditions of pH, ionic strength, and protein concentration for the purification of specific biomolecules need to be developed by the end user.

7.1 Working with i3 DMC^{CEX}

For optimal loading and elution with i3 DMC^{CEX} Membrane Adsorber, different buffer systems have been tested. It is recommended to use the same buffer system throughout the entire process. An addition of sodium chloride (NaCl) to the working solutions is recommended to increase the efficiency of the DMC elution. Recommended working solutions for i3 DMC^{CEX} are shown in the table below.

Buffer	Concentration	pH	Added electrolyte
Ammonium acetate	0.15 mM	6.1	0.15 mM NaCl
HEPES	0.1 mM	7.1	0.15 mM NaCl
MES	0.5 mM 0.1 mM	6.1 5.2	- 0.15 mM NaCl
MOPS	0.5 mM 0.1 mM	6.5 6.6	- 0.15 mM NaCl
PBS	0.24mM* 0.12 mM 0.012 mM	7.4 6.9 6.8	- - 0.15 mM NaCl

Table 1:
Recommended operating working solutions for i3 DMC^{CEX}

During DMC elution with the i3 DMC^{CEX}, pH values between 7 and 10 may occur. Immediately neutralize the samples by adding a small volume of a suitable neutralizing buffer. Depending on the further process, use buffer solutions such as PBS or ammonium acetate.

7.2 Recommended starting method

We recommend performing a bind/elute experiment using a purified negatively charged analyte. This serves to verify the correct functionality of the system and is preferably used for determination of DBC.

For loading and elution, a working solution of 0.24 mM PBS**, pH 7.4 is recommended.

The following steps demonstrate i3 standard bind/elute experiment with a flow rate of 2 mL/min.

Step	Action
1	Equilibrate the Membrane Adsorber with 3 mL of operation buffer. Make sure the UV baseline, pH and conductivity are stable
2	Load 5 mL of the sample with operating buffer
3	Wash with 7 mL of operating buffer
4	Elute with 4 mL of operating buffer and recommended voltage of +2 V
5	Wash membrane with 4 mL operating buffer.

A typical chromatogram for three bind/elute cycles of purified Lysozyme with DMC is shown below.

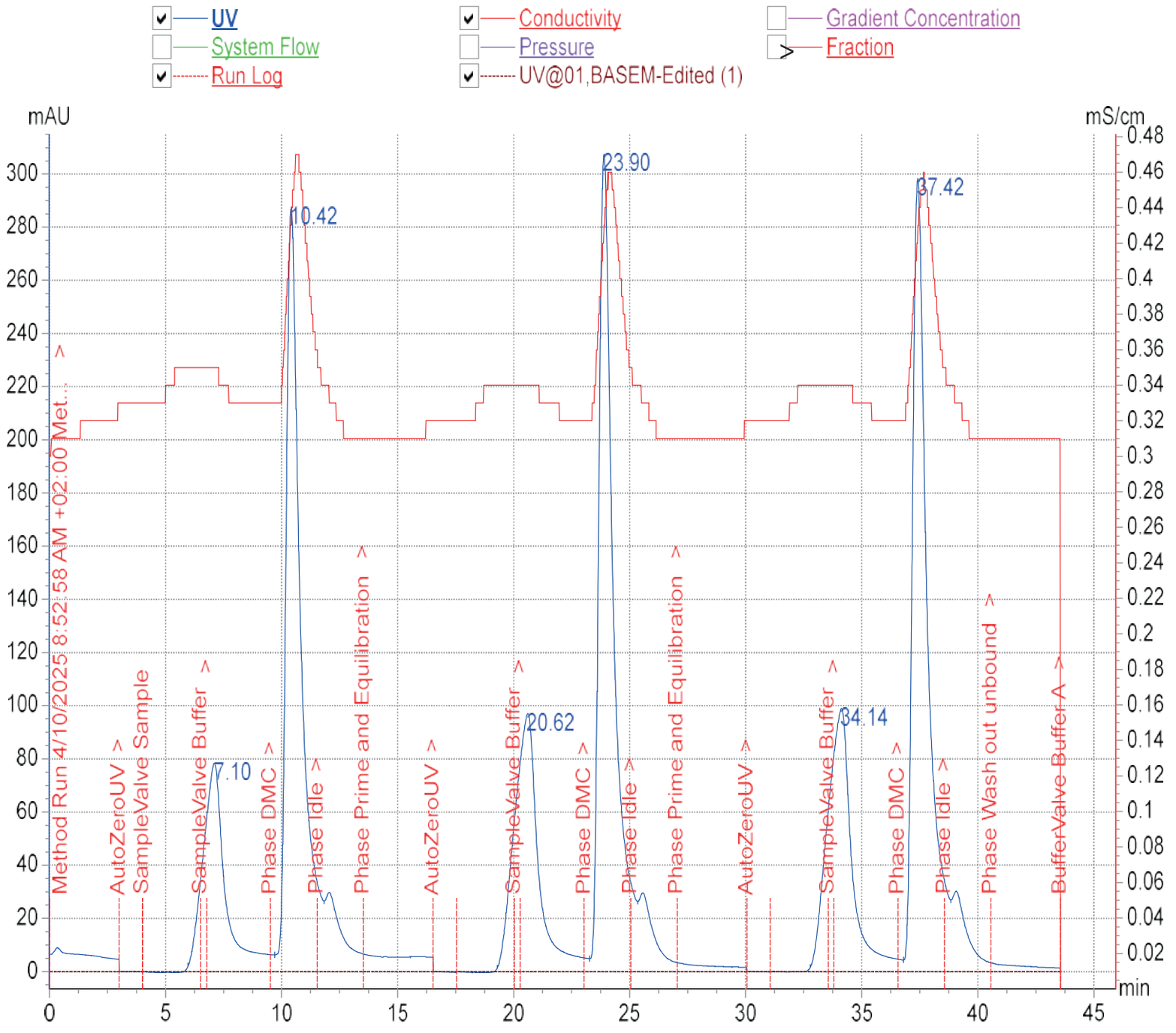


Figure 1: Bind/elute of Lysozyme using i3 DMC^{CEX}.
Three bind/elute cycles of Lysozyme (1.5mg/mL) in working solution 0.24 mM PBS**, pH 7.4 through DMC elution with i3 DMC^{CEX}. Initial sample concentration was 358.9 min*mAU at 280 nm (10 mm flow cell). DMC elution was done with +2 V.

8 Storage

It is recommended to connect the Membrane Adsorber to the FPLC only once for DMC purification runs. Incomplete post-run cleaning during storage can

9 Disposal

Dispose i3 DMC^{CEX} with household trash or according to the local guidelines.

10 Related documents

In addition to this instruction, related documents can be found at www.i3membrane.com/en/biotech

NOTES

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i3 Membrane GmbH | Global
biotech@i3membrane.de

i3 Membrane Corporate | U.S.
support@i3membrane.com

Visit our website at
www.i3membrane.com

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