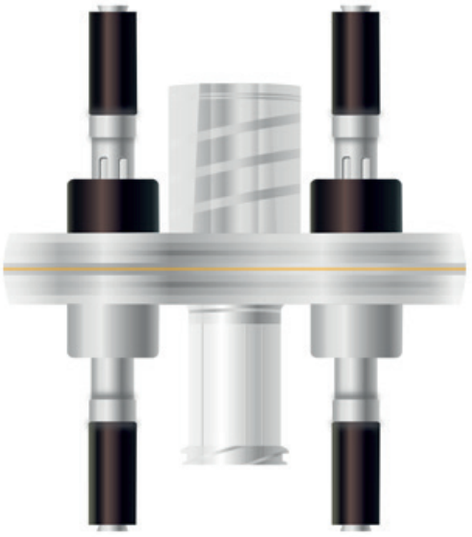
Instruction for use

i3 DMC^{PrA}

Protein A Membrane Adsorber



REF DMC401

Your Companion

Life Science Separation

Table of contents

1 Intended use

The i3 DMC^{PrA} is intended for the affinity purification of monoclonal antibodies and other Fc-containing proteins using electrical 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^{PrA} Membrane Adsorber can be operated with a peristaltic pump or a chromatography system.

2 Application

1. Ideal for rapid capture and release of highly concentrated monoclonal antibodies and Fc-containing molecules.
2. Enables working in small scale applications.
3. 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.
6. Wear protective working clothing, safety gloves and safety glasses.

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:	Cellulose
Pore size:	0.2 µm
Effective filtration area:	Ø 22.0 mm | 0.89"
Membrane thickness:	0.25 mm ± 30µm | 0.01" ± 0.0012"
Bed volume:	0.1 mL
Dynamic binding capacity per unit*:	Up to 5.1 mg IgG1
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: 0.24mM phosphate buffer, 2.74 mM NaCl and 0.054 mM KCL

6 Installation

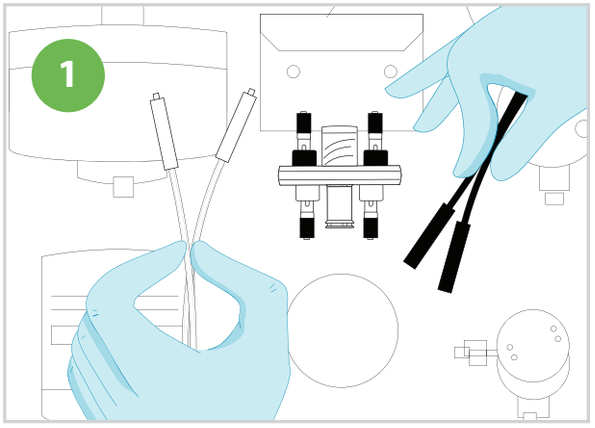
To install the i3 DMC^{PrA} Membrane Adsorber, you will need a liquid chromatography system (LC) with a Luer Lock connector. Additionally, when utilizing the DMC elution method, it is essential to have the i3 DMC$_{Control}$ Smart Control Unit with uniquely designed connecting cables.

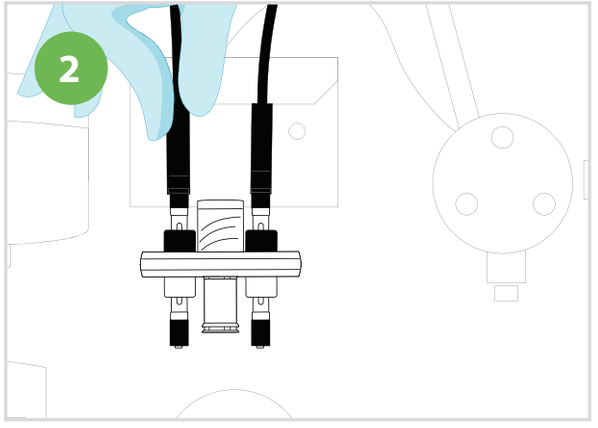
6.1 Installation of i3 DMC^{PrA}

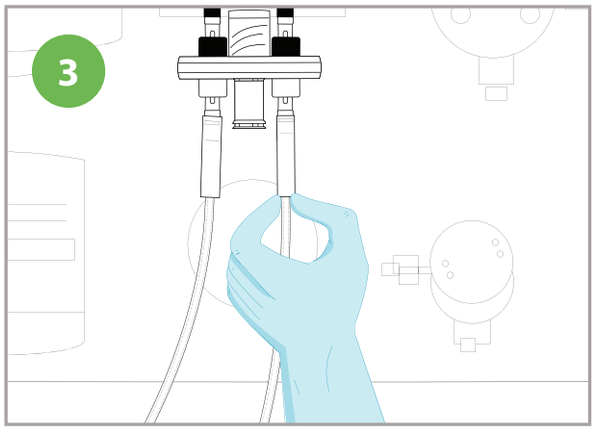
Follow the detailed step-by-step assembly instructions for installation of i3 DMC^{PrA}. Ensure that the membrane adsorber is securely connected to the i3 DMC$_{Control}$ and LC system. Loose connections may lead to suboptimal performance or device malfunction.

6.1.1 Installation to i3 DMC$_{Control}$

Utilizing the DMC elution method, it is essential to have the i3 DMC$_{Control}$ with appropriate connecting cables. Following steps describe the connection of i3 DMC$_{Control}$ and with uniquely designed connecting cables for i3 DMC^{PrA}. 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$_{Control}$.

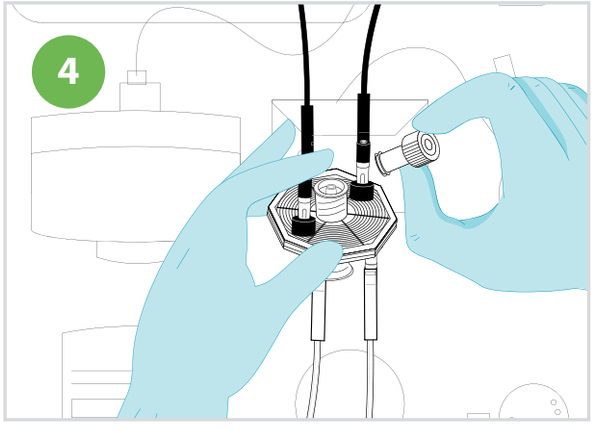


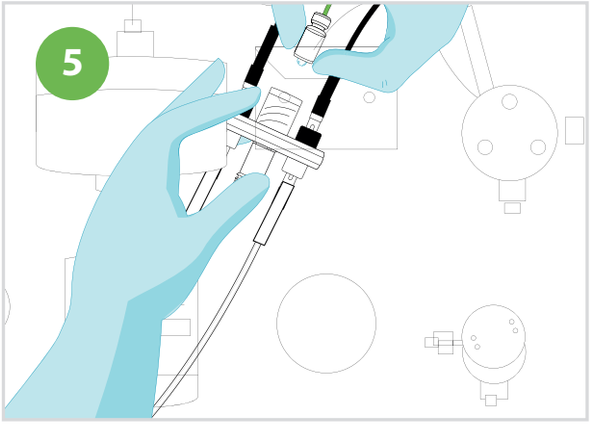


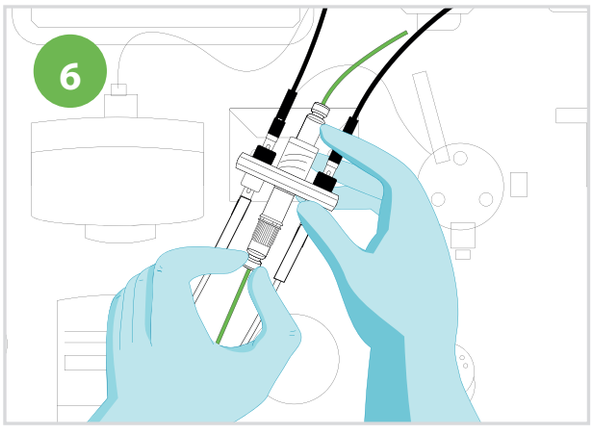


6.1.2 Installation to liquid chromatography (LC) systems

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.





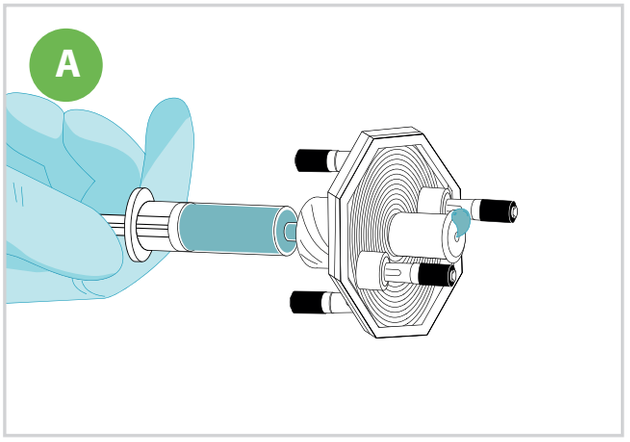


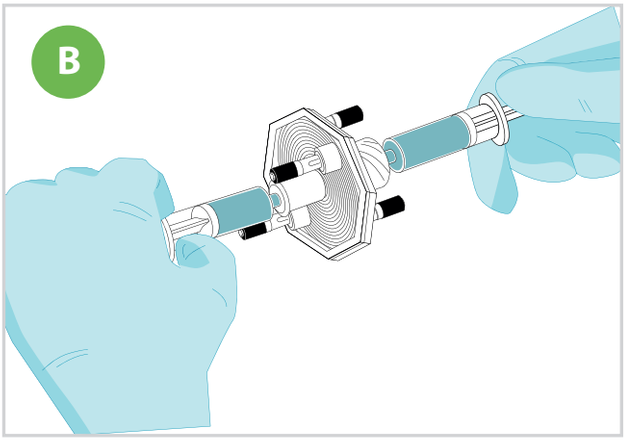
6.1.3 Venting

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^{PrA} 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 DMC elution

During DMC elution, an electrical potential is applied to the chromatography membrane to induce desorption of target proteins. Ensure that the electrical potential is applied exclusively during the elution phase. Application of voltage outside of the designated elution step may lead to process deviations or undesired effects on membrane performance.

For optimal elution efficiency, the addition of 0.5 mM sodium chloride (NaCl) to pH-neutral elution buffer is recommended. Further information on tested buffer systems can be found in FAQ section on our website. During DMC purification with the i3 DMC ^{PrA}, 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 IgG1. This serves to verify the correct functionality of the system and is preferably used for determination of DBC.

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

For subsequent measurements, loading can also be performed directly from pre-filtered cell suspension. DMC elution should be performed with 0.24 mM PBS ** pH 7.4 working solution.
The following steps demonstrate i3 standard bind/elute experiment with a flow rate of 2 mL/min.

Step	Action
Equilibration	Equilibrate the Membrane Adsorber with 1.5 mL of operation buffer. Make sure the UV baseline, pH and conductivity are stable
Loading	Load 5 mL of 3 mg purified IgG1 with working solution
Wash	Wash with 4.5 mL working solution
DMC elution	Elute with 4 mL of working solution and recommended voltage of +2V
Wash	Wash membrane with 6 mL of working solution.

A typical chromatogram for three bind/elute cycles of IgG1 with DMC is shown below.

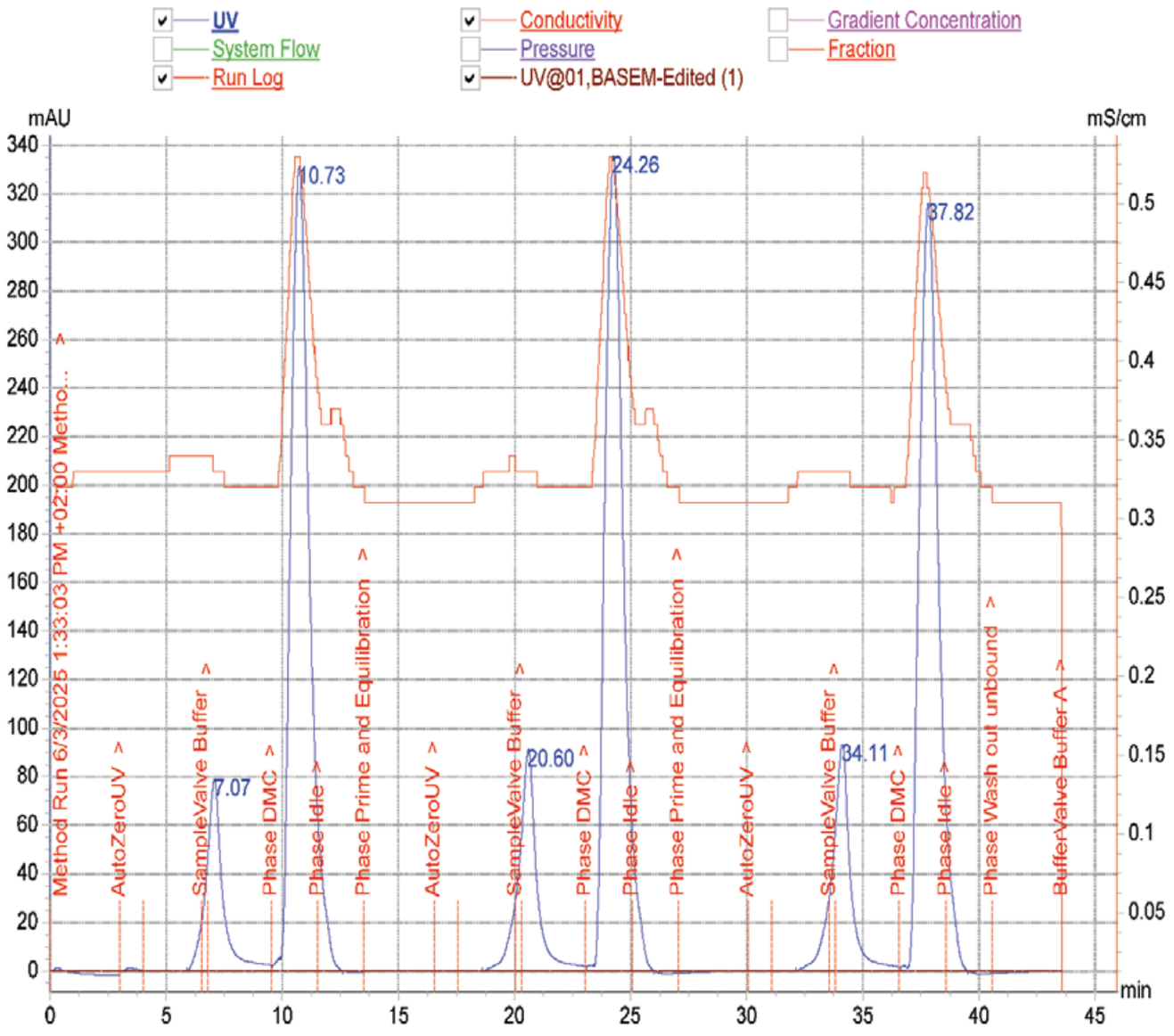


Figure 1: Three bind/elute cycles of hIgG1 using i3 DMC^{PrA}.
Bind/elute of Trastuzumab (3.0 mg/mL) in standard working solution 0.24 mM PBS**, pH 7.4 through DMC elution with i3 DMC^{PrA}. Initial sample concentration was 421.92 min*mAU at 280nm (10 mm flow cell). DMC elution was done with +2V.

7.3 Cell culture supernatants

For the purification of cell culture supernatants, it is essential to clarify the material by conventional centrifugation followed by 0.22 µm sterile filtration. The presence of particulates may clog the membrane adsorber and lead to an increase in back pressure. If membrane clogging occurs, backflushing of the membrane followed by a CIP procedure (see section 8) is recommended. Multiple DMC purification cycles and reusability of the Membrane Adsorber depend strongly on the individual cell culture supernatant.

The sample can be loaded directly from the pre-filtered cell culture supernatant onto the Membrane Adsorber for purification. Ensure that the i3 elution working solution with the recommended NaCl additive is used for the wash and elution step. Do not exceed the conductivity of the recommended working solution, as higher conductivity will reduce the efficiency of the DMC elution process.

8 Cleaning-in-Place (CIP)

Strongly bound impurities, such as host cell proteins (HCPs), lipids, and other particulates, can cause membrane clogging. This can cause decreasing capacities and recovery. Reversed flow can be applied to efficiently remove impurities that lead to rapid filter blockage.

It is recommended to flush the membrane adsorber with ultrapure water until the transmembrane pressure has decreased and the UV signal returns to baseline levels.

In addition, the following substances and procedures can be employed for membrane cleaning without adversely affecting its mechanical or electrical properties.

- 0.1 M NaOH
- 0.1 M Citrate Buffer
- $NaHCO_3$
- 10x alternating voltage pules of +2V and -2V, each applied for 1 sec.

The effectiveness of the chemical cleaning procedure depends on the specific characteristics of the cell culture supernatant used. The suitability and performance of the applied chemical treatment must be validated for each individual application.

9 Storage

After DMC purification, flush the unit extensively with ultrapure water to remove residual buffer components before disconnecting it from the system. For subsequent reuse, store the Membrane Adsorber in 20% ethanol/water at 2–8 °C. Prior to the next chromatographic run, reinstall the Membrane Adsorber and equilibrate with ultrapure water. Residual electrolytes within the membrane device may cause the DMC control unit self-test to fail. If the self-test remains unsuccessful after thorough equilibration, install a dummy adsorber to pass the self-check. Thereafter, prepare the Membrane Adsorber by applying recommended voltage without sample. Subsequently, a new purification cycle with the membrane adsorber can be initiated.

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

10 Disposal

Dispose i3 DMC[PrA] with household trash or according to the local guidelines.

11 Related documents

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

NOTES

Your Companion

Life Science Separation

i3 Membrane GmbH | Global
biotech@i3membrane.de

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

Visit our website at
www.i3membrane.com

Your Companion

Life Science Separation

IFU_i3DMC_PrA_EN_
AUG2025_V01