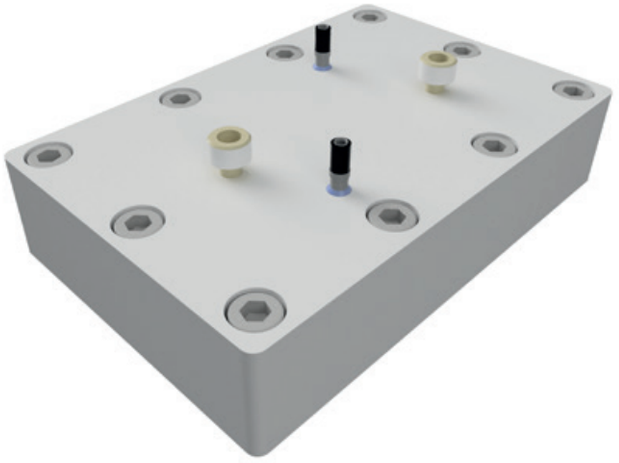
Instruction for use

i3 DMC^{PrA} 1.0 mL

Protein A Membrane Adsorber



REF DMC402

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1 Intended use

The i3 DMC^{PrA} 1.0 mL is a ready-to-use membrane adsorber for rapid affinity purification of antibodies. Its Protein A functionalized membrane efficiently captures immunoglobulin G (IgG), monoclonal antibodies (mAbs), and other Fc-containing molecules with high specificity for general laboratory applications and early-stage research and development.
With its electrically driven elution, the DMC technology enables purification without the use of low-pH elution buffer and delivers samples in a physiological buffer suitable for direct developability assays. The device can be operated with any conventional liquid chromatography (LC) system.
For research use only. Not intended for use *in vitro* diagnostic or therapeutics procedures.

2 Application

1. Mild affinity purification of pH-sensitive antibody modalities.
2. No buffer exchange.
3. Antibody discovery.

3 Safety instructions

1. Do not use the filter outside of its specifications.
2. Do not use organic solvents.
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 sterile 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
Ligand:	Protein A
Pore size:	0.2 µm
Membrane dimensions:	76.5 x 32.5 mm
Membrane thickness:	0.625 mm ± 30µm | 0.025" ± 0.0012"
Bed volume (BV)/Membrane volume (MV):	1.0 mL
Dynamic binding capacity (DBC)/unit[1]:	Up to 30 mg polyclonal IgG
Elution volume:	8 mL (8 MV)
Recommended operating flow rate:	3.0 mL/min (3 MV/min)
Maximum operating pressure:	Up to 5 bar | 72.5 psi
Recommended elution voltage:	+3.35 V
Temperature stability:	4° C-RT | 39.2° F-RT
Cleaning-in-place (CIP):	Up to 10x with 0.1 M NaOH, short-time incubation
Connectors:	PEEK Fingertight- Fittings,10-32 thread
Autoclavability:	Non autoclavable
Storage:	20% ethanol/water, 4°C | 39.2°F
Dimensions:	L: 130.0 mm | 5.1" W: 86.0 mm | 3.4" H: 28.0 mm | 1.1"

[1] Determined in 1:50 diluted 1x PBS (containing 2.74 mM NaCl, 54 µM KCl, 0.24 mM phosphate buffer, pH 7.4)

6 Installation of i3 DMC^{PrA} 1.0 mL

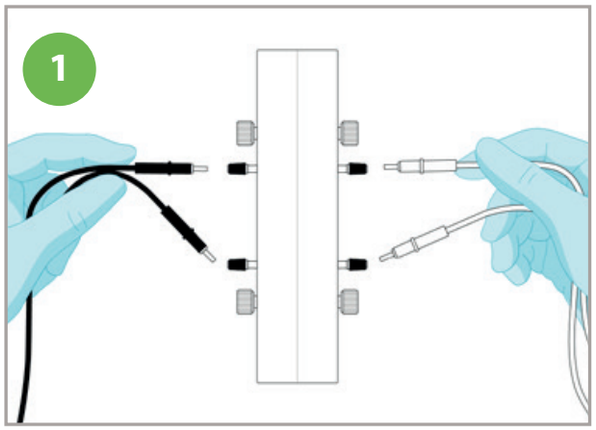
To install the i3 DMC^{PrA} 1.0 mL Membrane Adsorber, you will need a liquid chromatography system (LC) with a PEEK Fingertight-Fittings, 10-32 Thread and two large syringes for venting. Additionally, for utilizing the DMC elution method, it is essential to have the i3 DMC$_{Control}$ Smart Control Unit with uniquely designed connecting cables.

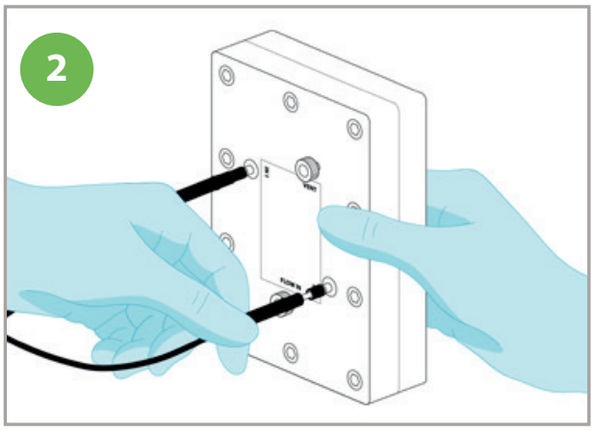
Follow the detailed step-by-step assembly instructions for installation of i3 DMC^{PrA} 1.0 mL. Ensure that the membrane adsorber is securely connected to the i3 DMC$_{Control}$ and the LC system. Loose connections may lead to suboptimal performance or device malfunction.

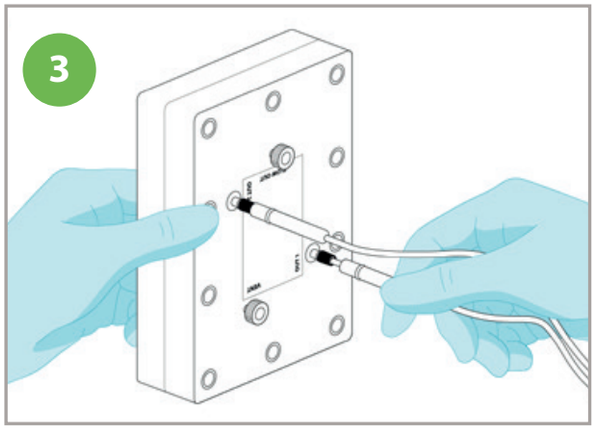
6.1 Connecting to i3 DMC$_{Control}$

The i3 DMC$_{Control}$ operates as a standalone device and is not connected to the LC system. Only the membrane adsorber is connected to the LC system. The i3 DMC$_{Control}$ connects directly to the membrane adsorber using the designated black and white connection cables. Following steps describe the connection of the i3 DMC$_{Control}$ to the i3 DMC^{PrA} 1.0 mL membrane adsorber.

Place the device near the membrane adsorber to facilitate operation and avoid excessive tension of the connection cables. Turn on the i3 DMC$_{Control}$ and connect a dry membrane adsorber before initiating the self-check procedure. Identify the appropriate connection cables (picture 1). Connect the corresponding inlet and outlet contact pins of the membrane adsorber, ensuring that the black inlet cables are connected to the inlet pins IN1 and IN2 (picture 2) and the white outlet cables are connected to the outlet pins OUT1 and OUT2 (picture 3). Ensure that all cable connections are securely attached. Verify the correct cable assignment: black to IN1/IN2 and white to OUT1/OUT2. Do not interchange the connections. To perform experiments with the membrane adsorber and i3 DMC$_{Control}$, follow the operating instructions provided in the i3 DMC$_{Control}$ user manual.







6.2 Venting procedere

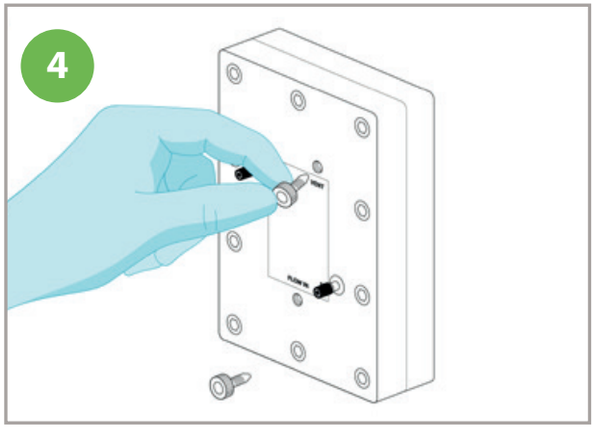
Venting of the i3 DMC^{PrA} 1.0 mL membrane adsorber is required to ensure complete wetting for proper operation. During the venting process, do not exceed a pressure of 5 bar (0.5 MPa | 72.5 psi), as excessive pressure may damage the membrane or the filter housing.

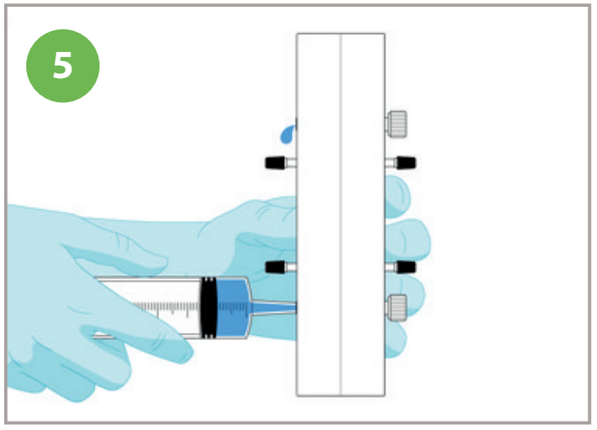
The membrane adsorber is equipped with a flow inlet on the front side (FLOW IN) and a flow outlet on the rear side (FLOW OUT). Both sides are equipped with a vent port. Ensure that the unit is vented from both sides.

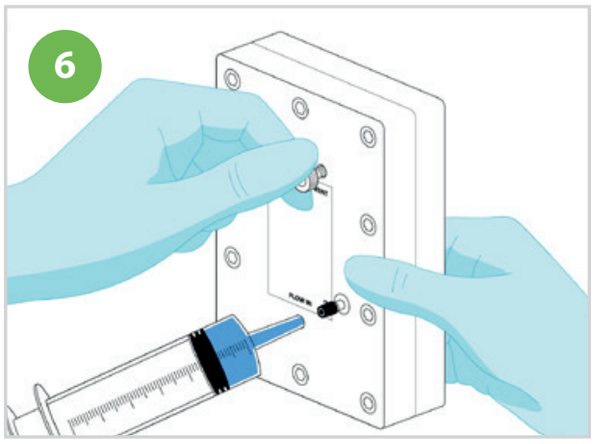
Fill two syringes with buffer and ensure that no air is trapped in the syringe. Remove the plugs from the flow inlet and the vent port on the front side (picture 4). Connect a syringe to the flow inlet of the membrane adsorber. Gradually fill the unit until a drop of buffer appears at the open vent port and no more air bubbles are expelling (picture 5). Once venting of the front side is completed, close the vent port using the plug (picture 6).

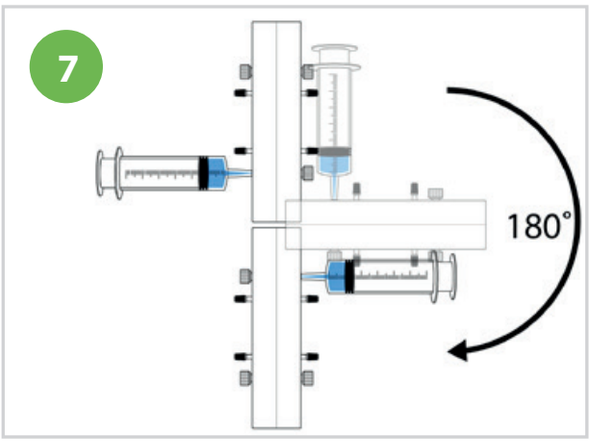
Rotate the device by 180° to vent the rear side of the membrane adsorber (picture 7). Remove the plugs from the flow outlet and the vent port on the rear side. Connect the second syringe to the flow outlet and fill the unit using the outlet syringe until buffer drips out of the open vent port (picture 8). Close the vent port with the plug once no more air bubbles are observed.

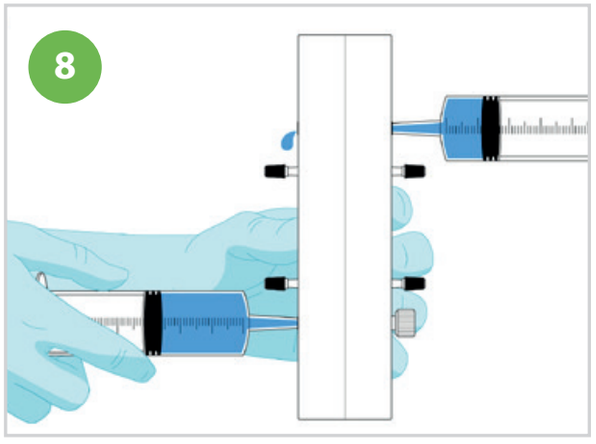
Start by alternately flushing the unit using both syringes to remove residual air trapped within the membrane structure and fluidic channels (picture 9). During this process, an increase in hydraulic resistance should be noticeable, indicating that the unit is becoming fully filled. Without applying too much pressure, carefully continue the alternating flushing procedure until no air bubbles are released from either side of the membrane adsorber. Disconnect the syringes and proceed with the connection to the liquid chromatography (LC) system.

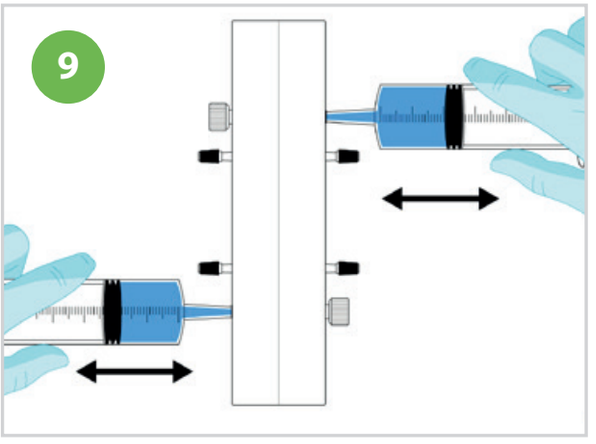






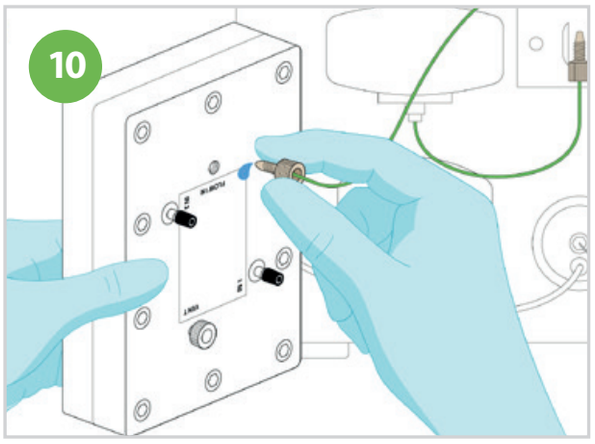


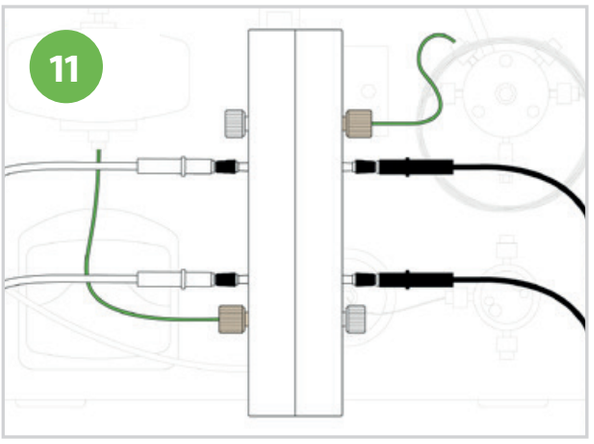




6.3 Connecting to liquid chromatography (LC) systems

For the operation of the membrane adsorber with an LC system, remove the plugs of the flow inlet (FLOW IN) and flow outlet (FLOW OUT) of the membrane adsorber and start your fluid flow. Once the buffer begins to emerge, connect the tubing to the flow inlet of the membrane adsorber using a PEEK Fitting (picture 10). Wait until the buffer drips out of the flow outlet of the membrane adsorber and connect the flow outlet to the tubing to the LC system (picture 11). Use a tissue to dry the outer surface of the unit from excessive liquid after the filling process.





7 Operation

To ensure efficient purification using i3 DMC^{PrA} 1.0 mL Membrane Adsorber with DMC elution method, follow the procedures outlined in the subsequent sections. Application-specific protocols 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 Preparations

7.1.1 Sample preparation

Before purification with DMC, samples should be sufficiently clarified to prevent membrane clogging. For cell culture supernatants, clarification by conventional centrifugation followed by 0.22 µm sterile filtration is recommended. The presence of unfiltered particles 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. The ability to perform multiple DMC purification cycles and the reusability depend strongly on the individual sample composition.

7.1.2 Buffer preparation

For all steps we recommend using a 1:50 diluted 1x PBS buffer (containing 2.74 mM NaCl, 54 µM KCl, 0.24 mM phosphate buffer, pH 7.4), hereafter referred to as working solution.

Pre-filtered samples may be loaded directly onto the membrane adsorber without the need of buffer exchange. After sample loading, the membrane must be washed thoroughly with the working solution to reduce the conductivity to approximately < 0.5 mS/cm before DMC elution. Do not exceed the conductivity of the recommended working solution, as higher conductivity will reduce the efficiency of the DMC elution process. Further information on tested buffer systems can be found in FAQ section on our website.

During DMC purification with the i3 DMC^{PrA} 1.0 mL, pH values between 7 and 10 may occur. The collected elution typically has a physiological pH. However, if the elution is collected in fractions, make sure to adjust the pH of the fractions by adding a small volume of a buffered solution. For long-term storage of the collected samples, keep the sample in a buffer suitable for subsequent analyses.

7.2 Working wit 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.

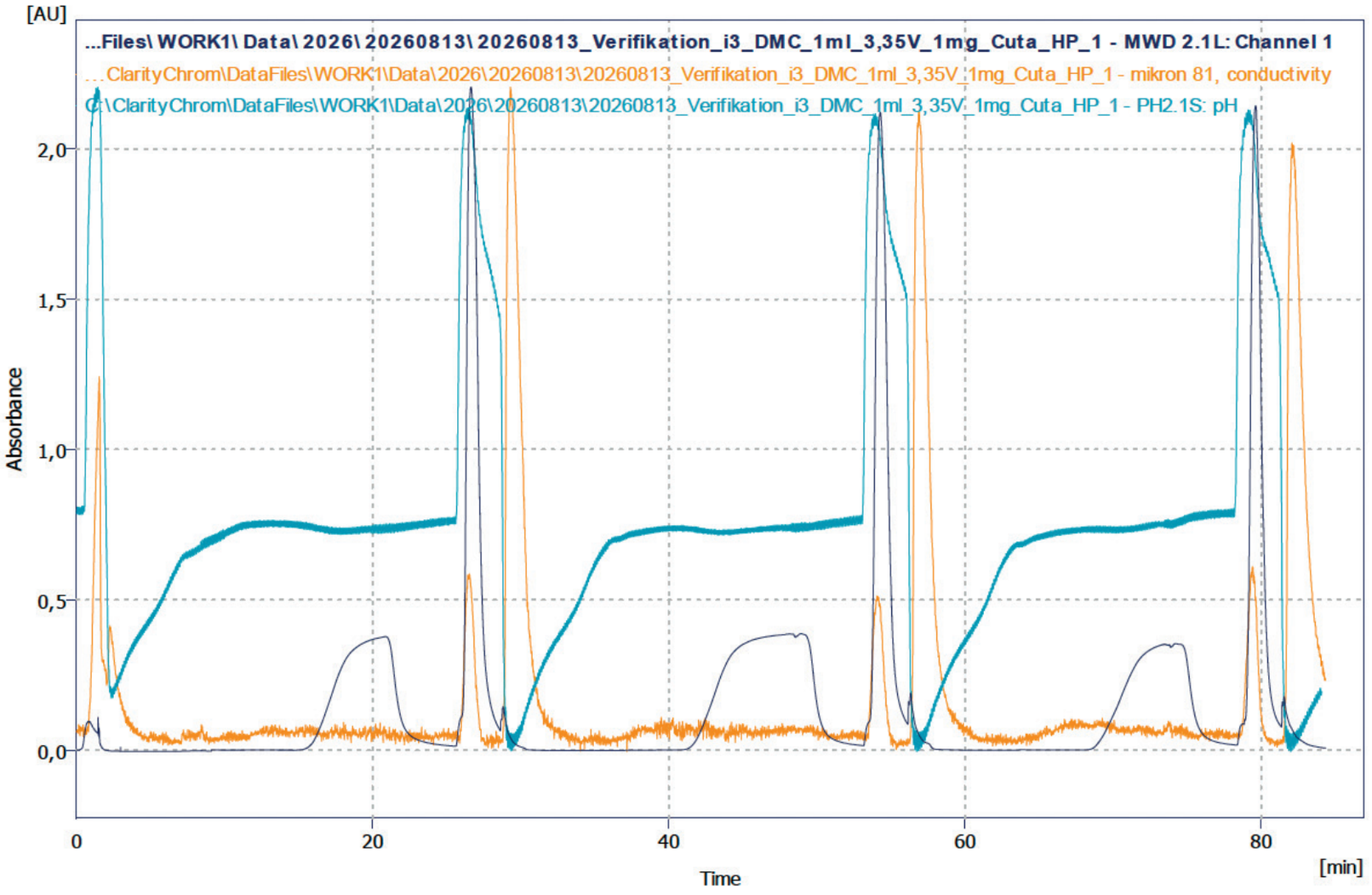
7.3 Recommended starting procedure

We recommend an initial performance test in bind/elute mode using a purified IgG antibody to verify the system's correct function and to confirm the dynamic binding capacity (DBC).

The following steps demonstrate i3 standard bind/elute experiment with a polyclonal IgG (cutaquig®) at a flow rate of 3 mL/min.

Step	Action
Equilibration	Equilibrate the membrane adsorber with working solution. Make sure the UV baseline, pH and conductivity are stable.
Loading	Load at total of 30 mg purified IgG diluted in 30 mL working solution.
Wash	Wash with working solution. Make sure the UV returns near to baseline.
DMC elution	Elute in 8 mL working solution by applying a single voltage step at + 3.35 V for 3 min.
Wash	Wash with working solution. Before performing a next cycle make sure the pH returns to approx. pH 7.

It is important that the manually initiated elution at the $DMC_{Control}$ is triggered simultaneously with the start of the elution on the LC system. For easier handling we recommend programming a pause in the LC method immediately before the elution step. This ensures that the elution is started at the same time on both the LC system and the $DMC_{Control}$.
A typical chromatogram of three bind/elute cycles of polyclonal IgG using DMC is shown below (Figure 1).



blue: UV **orange: Conductivity** **petrol: pH**

Figure 1: Performance of i3 DMC^{PrA} 1.0 mL in three consecutive capture and release experiments.
30 mg polyclonal IgG (cutaquig®) was loaded onto i3 DMC^{PrA} 1.0 mL membrane adsorber resulting in a breakthrough peak caused by the overload. After washing, elution was performed in working solution by applying +3.35 V for 3 min. Recovery rates of > 93% were obtained with high reproducibility.

8 Cleaning-in-Place (CIP)

Strongly bound impurities, such as host cell proteins (HCPs), lipids, and other remnants, can cause membrane clogging. This can decrease the binding capacity and sample 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 decreases and the UV signal returns to baseline levels.

In addition, 0.1 M NaOH can be utilized up to ten times for membrane cleaning without compromising its mechanical or electrical properties. The effectiveness of the chemical cleaning procedure depends on the specific characteristics of the sample used. The suitability and performance of the chemical treatment must be validated for each individual application.

9 Storage and Reuse

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 4°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 i3 $DMC_{Control}$ unit self-test to fail. If the self-test remains unsuccessful after thorough equilibration, install a dry dummy adsorber to pass the self-check. Subsequently, a new purification cycle with the membrane adsorber can be initiated.

10 Disposal

Dispose i3 DMC PrA 1.0 mL with household trash or according to the local guidelines.

11 Related documents

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

NOTES

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