GORE® Protein Capture Devices for Drug Discovery Applications

1.0 mL and 3.5 mL

Operating Instructions

Contents

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Product Description

The GORE Protein Capture Devices in 1.0 mL and 3.5 mL are intended for affinity purification of antibodies from clarified feed streams in drug discovery applications and in the production of antibodies for research and development work. The Devices contain a membrane bed with immobilized Protein A that provides a binding capacity advantage to improve the speed of purification. In addition, GORE Protein Capture Devices yield highly concentrated protein, which may eliminate the need for a downstream concentration step. These unique membrane devices are offered in 1.0 mL (≥30 mg capacity) and 3.5 mL (≥105 mg capacity) sizes (Figure 1).

Figure 1. GORE Protein Capture for Drug Discovery Applications

1.0 mL GORE Protein Capture Device (PROA101)

3.5 mL GORE Protein Capture Device (PROA102)

Intended Use

GORE Protein Capture Devices (1.0 mL and 3.5 mL sizes) are intended for research use only and should not be used for clinical or diagnostic procedures.

Device Characteristics

Table 1. GORE Protein Capture Device Characteristics

Nominal Bed Volume	DBC*	Recommended Flow Rate	Capacity for IgG at 30 mg/mL DBC	CIP Stability	Maximum operating pressure limit	pH range
1.0 mL	≥ 30 mg/mL	3.0 mL/min	30	See CIP section	5 bar (0.5 MPa)	2–13
3.5 mL		10.5 mL/min	105			

*Dynamic binding capacity is determined using human polyclonal IgG at 10% breakthrough with a residence time of 20 seconds. In addition, the 1.0 mL devices were tested with the flow restrictor in-line on the liquid chromatography (LC) system.

DBC dynamic binding capacity; CIP clean-in-place; IgG immunoglobulin G; NaOH sodium hydroxide; MPa megapascal

General Handling

Flow Direction

The GORE Protein Capture Devices can be operated with flow running in either direction. If pressure develops over time, the flow can be reversed and/or cleaning can be performed to reduce the operating pressure.

Flow Rate Selection

The GORE Devices have a dynamic binding capacity (DBC) of ≥30 mg/mL using 1.25 mg/mL human polyclonal IgG in phosphate-buffered solution at a residence time of 20 seconds, thus allowing for higher flow rates than traditional purification technologies. The flow rate is selected based on pressure. For example, at a residence time of 20 seconds, a 1.0 mL Device would have a flow rate of 3.0 mL/min, whereas a 3.5 mL Device would have a flow rate of 10.5 mL/min. See application note PB8433 for alternative flow rates to improve the productivity of purification.

LC System Recommendations

It is recommended to physically remove the flow restrictor or turn it to off-line in the software when running the 3.5 mL Device. The flow restrictor may be used with the 1.0 mL especially if encountering issues with air in the system. It is recommended to replace the waste line with 3/16" diameter tubing. For ideal conditions, it is recommended to plumb system with 0.75 mm ID green PEEK tubing.

Buffer And Solutions

All liquid chromatography solutions including, but not limited to, water, chemical solutions, and protein solutions should be highly pure, thoroughly dissolved, and filtered through a 0.2 µm filter to remove particulates, solids, and other large-size impurities.

Protection Of Purification Device

Protein solutions should be highly pure, thoroughly dissolved, and filtered through a 0.2 µm filter to remove particulates, solids, and other large-size impurities. Impurities, protein agglomerates, and particles can play a role in the performance of the affinity chromatography device, especially if the harvest stream or target antibody is known to be susceptible to agglomeration and/or particulates. It is recommended to use an inline 0.2 µm guard column to protect the Device, especially if the feed stream or target antibody is known to be susceptible to agglomeration and/or particulates.

A maximum pressure setting of 0.5 MPa (5 bar) is recommended to avoid potential damage to the Device. The Device is designed with 10-32 threaded fittings. The end plugs are threaded and should not be pulled-out, pushed-in or over-tightened to avoid damage to the threads. The user should refer to the Product Safety Sheet for specific handling procedures.

Antibody Purification Protocol

Optimization of binding and elution buffers may be dependent on the harvest stream and antibody and will be specific to the customer. Additional buffers that have been tested include phosphate-buffered solution and glycine, citrate, and Tris buffers.

Table 2. Example buffers for antibody purification using GORE Protein Capture Devices

Solution/Buffer	Chemical Composition	pH	Conductivity (mS/cm)	Step
Phosphate- Buffered Solution	150 mM NaCl 50 mM Phosphate	7.4 ± 0.10	20±1.0	Column equilibration Binding equilibration
Citrate Buffer	100 mM Citrate	3.4 ± 0.20	4.9±1.0	Elution buffer
Sodium Hydroxide	0.1 N NaOH	13.0 ± 0.30	21±1.0	Clean-in-Place solution
DI Water	H_2O	N/A	N/A	Used for all buffers and aqueous solutions
Ethanol (200 proof)/ DI Water Solution	20%/80% (v/v)	N/A	N/A	Buffer for long-term storage of the protein affinity Device

DI = deionized; mS/cm = millisiemens per centimeter; mM = millimolar; N = Normal; N/A = not applicable; NaCl = sodium chloride; NaOH = sodium hydroxide; v/v = volume/volume

Sample preparation

The pH of the feed stream should be adjusted based on the properties of the antibody to be purified to ensure maximum binding to the membrane column. In addition, the clarified feed stream should be filtered through a 0.2 µm filter to reduce debris and particulates. If the feed is prone to aggregation, it is highly recommended to perform depth filtration for further clarification of the feed.

Running Conditions

Note: Gore Devices are shipped in 20% (v/v) ethanol in deionized water. The Device should be rinsed with deionized water before initial use. It is recommended to flush the Device with water at a reduced flow rate for 5-10 column volumes (CV); slowly increase the flow rate until pressure stabilizes.

If operating at temperatures lower than room temperature (ex. 2-8°C), the pressures observed may be higher and reduce performance of the Device due to increased viscosity of solutions.

Purification with Clean-in-Place protocol

Note: A clean-in-place (CIP) cycle should be performed using sodium hydroxide (NaOH) after each cycle. See CIP section for recommendations based on number of cycles desired.

Note: Flow rates for the purification steps provide a residence time of 20 seconds (3.0 mL/min for a 1.0 mL device and 10.5 mL/min for a 3.5 mL device) unless stated otherwise. In addition, a longer residence time may increase binding capacity. The user can refer to the data sheet (PB6575) for comparison data at various residence times.

Note: A pre-conditioning run, including a CIP step, may be performed for new Devices and for Devices following long term storage (>/= 1 month).

Note: Differences in the harvest source or harvesting conditions may affect purification of the antibody. If an optimization of the purification protocol is needed, consider optimizing sample preparation, buffers' composition and pH, residence time, washing protocol, and/or elution protocol.

1. Remove the Device from the packaging and wash with deionized water using at least five column volumes.

   Note: Reduced flow rates used in this step will prevent pressure spikes and bubble formation.

   a. Wash with 5.0 mL at a flow rate of ≤ 1.0 mL/min for a 1.0 mL Device.
   b. Wash with 17.5 mL at a flow rate of ≤ 3.5 mL/min for a 3.5 mL Device.

2. Equilibrate the Device with ten column volumes of phosphate-buffered solution.

3. Load the device with the feed containing the target antibody.

   Note: Gore verified the continued performance of the Device for at least 20 purification/cleaning cycles by using clarified and filtered extracts harvested from CHO cells.

   a. Determine sample loading by using one of the following recommendations:
      i. Load the sample according to the DBC provided on the Certificate of Analysis
      ii. Load the sample according to the 10% breakthrough determined from an overload cycle performed prior to purification

4. Wash the device with ten column volumes of phosphate-buffered solution to remove loosely bound material.

   Note: Specific wash protocols can be created and/or optimized based on the properties of the antibody being purified and the purity of the feed stream.

5. Elute the antibody from the device with three to six column volumes of citrate buffer using a 100% isocratic elution.

   Note: Gradient elution protocols can be created and optimized for individual antibodies if needed.

   Note: Eluted fractions can be neutralized with 1 M Tris buffer at pH 9. It is recommended to use between 250 and 300 µL of 1 M Tris buffer (pH 9) to obtain a neutral pH. The elution pH should be optimized to the isoelectric point of the target protein being purified. In addition, the amount of 1 M Tris buffer (pH 9) used for neutralization may vary depending on the isoelectric point of the target protein.

6. Wash the device with five column volumes of phosphate-buffered solution in the reverse direction of loading

7. Perform CIP/sanitization by running 0.1 N sodium hydroxide for 15 minutes of contact time at a flow rate at 1.0 mL/minute. For an alternative cleaning method to extend the lifetime, see the CIP section and application note, PB8850.

   Note: The customer can refer to the CIP protocol section for additional information.

8. Wash the Device with ten column volumes of phosphate-buffered solution to equilibrate to a neutral pH.
9. Rinse the Device with five column volumes of deionized water to remove salt.
10. Add five column volumes of 20% ethanol (v/v) in deionized water to the Device using the flow rate recommended in step 1 (5.0 mL at a flow rate of ≤ 1.0 mL/min for the 1.0 mL Device and 17.5 mL at a flow rate of 3.5 mL/min for the ≤ 3.5 mL Device).

    Note: Steps 9 and 10 can be disregarded if the Device is going to be used for multiple runs. These steps are performed after all purification runs are completed for the day and the Device is going to be stored.

Table 3 shows column volumes used per purification step for the 1.0 mL and 3.5 mL Devices at the recommended flow rates. An alternative method has been developed for higher productivity, see application note PB8433, Increasing the Productivity of mAb Purification using the GORE Protein Capture Devices with Protein A.

Table 3. Example of an Antibody Purification Procedure

#	Step	Buffer	Column Volumes	Residence Time (seconds)	1.0 mL Device Flow Rate (mL/min)	3.5 mL Device Flow Rate (mL/min)
1	Equilibration	DI Water	5	60	1.0	3.5
2	Equilibration	Phosphate-Buffered Solution	10	20	3.0	10.5
3	Load	Feed Stream	Determined by sample concentration	20	3.0	10.5
4	Wash	Phosphate-Buffered Solution	10	20	3.0	10.5
5	Elute	Citrate Buffer	Collection of elution peak[1]	20	3.0	10.5
6	Wash	Phosphate-Buffered Solution	5	30	3.0	10.5
7	Clean	0.1 N Sodium Hydroxide	Not Applicable[2]	Not Applicable[2]	1.0	1.0
8	Wash	Phosphate-Buffered Solution	10	50	3.0	10.5

[1] Collection was started at an absorbance of 100 mAU and continued until the absorbance returned to 100 mAU
[2] CIP with 0.1 N sodium hydroxide for 15 minutes at a flow rate of 1.0 mL/min. The CIP cycle can be performed as needed.
DI deionized; mAu milli absorbance unit

Clean-In-Place and Sanitization

A CIP cycle should be performed after each purification cycle using NaOH. The CIP can include a 0.2 M NaOH for 3 minutes contact per Gore application note PB8850, *Extending Lifetime of GORE Protein Capture Devices through Alternative Cycling Parameters*. It is recommended to perform a CIP cycle or sanitization with 0.1 M NaOH for 15 minutes at a flow rate of 1.0 mL/min. before switching to a new feed or purification protocol, or on the last run of the column prior to long-term storage.

Do not expose the device to prolonged contact with 0.1 N sodium hydroxide as this can irreversibly damage the device. Please follow the recommended contact times. In addition, do not expose the device to greater than 0.1 N sodium hydroxide.

Regeneration steps with 6 M guanidine hydrochloride or 6 M urea have been used in lieu of a CIP cycle. Optimization of this step will be dependent on the feed stream and will be specific to the customer.

Storage

Store the Device in 20% ethanol/ 80% deionized water at a temperature range of 2–8°C (35– 46°F). It is recommended to equilibrate the Device and perform a blank run, including CIP step, after storage and before use. If the Device is going to be or has been stored for an extended period of time, a sanitization should be run with 0.1M NaOH for 15 minute contact time.

Shelf Life

The GORE Protein Capture Device has a shelf life of one year (12 months) at 2–8°C (35– 46°F) under recommended storage conditions.

Labeling

Each GORE Protein Capture Device is given a unique identifier to enable traceability back to the master lot and raw material components. Each individual Device is labeled with part number and lot number. Each Device package is labeled with product name, part number, lot number, serial lot number, manufacture date, and expiration date.

Serial Use

GORE Devices can be connected in serial fashion to increase capacity when purifying larger amounts of antibody.

Ordering information

Part Number	Description	Quantity
PROA101	1.0 mL Device	1/each
PROA102	3.5 mL Device	1/each

Troubleshooting

Issue	Recommendation
Increase in backpressure	• Evaluate cleaning protocol (See Gore's application note number PB8850 on *Extending Device* lifetime for additional recommendations) • Optimize sanitization cycle • Perform one or more CIP cycles as follows: – Perform a CIP cycle in the same direction used for loading – Check the back pressure after the CIP cycle – Reverse flow direction and perform another CIP cycle if back pressure remains high – Include an acid strip after elution – Refer to Gore application note number PB1502 *Cleaning Protocols to Reduce the Effects of Pressure Rise over the Lifetime of a GORE Protein Capture Device* for optional cleaning procedures
Leakage at fittings	• Tighten the 10-32" fittings and ensure proper alignment with the threads
Loss of capacity over time	• Verify DBC and load antibody to the recommended loading volumes (70 to 90% of the 10% breakthrough) • Ensure Device undergoes a CIP cycle after each purification cycle • Perform sanitization with 0.1N NaOH for 15 minutes
Protein A leaching	• Protein A leaching occurs with many affinity devices; the GORE Device is designed to ensure that leaching per cycle during the life of the device (up to 20 cycles) is ≤100 ppm. • Can be antibody dependent and may require additional optimization of the purification protocol to meet specific leaching requirements
HCP/other impurities in elution pool	• Follow recommendations below: – Load the feed or extract – Perform an optional wash step using a buffer with high salt concentration (0.5 to 1.0 M NaCl at pH≥7.0) – Perform the elution in the reverse direction to remove noncovalently-bound contaminates
Elution peak tailing	• If possible, lower pH of the elution buffer • If possible, increase the ionic strength of the elution buffer
Air bubbles Recommendation	• Run column in reverse • Ensure solutions and tubing lines are free of air bubbles, use bypass at higher flow rates to eliminate air bubbles from the lines prior to running through the Device • Ensure the Device isn't tilted during flow • Check equipment filters and sensors for air or clogging

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PB6576 Rev4 MAR20 P/N 10949569