GORE® Protein Capture Device
9.0 mL

Operating Instructions

Contents

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Product Description

The GORE Protein Capture Device - 9.0 mL (Figure 1) is intended for affinity purification of antibodies from clarified harvest streams in process development and clinical applications. The Devices contain a bed with immobilized Protein A. The bed is made of a unique expanded polytetrafluoroethylene (ePTFE) membrane composite that provides a binding capacity advantage at high flow rates (Table 1) and thereby improves the speed of purification.

Figure 1. GORE Protein Capture Device – 9.0 mL

Intended Use

GORE Protein Capture Devices, 9.0 mL are intended for clinical applications and process development.

Device Characteristics

Table 1. GORE Protein Capture Device Characteristics

Nominal Bed Volume	DBC* (mg/mL)	Recommended Flow Rate (mL/min)	Capacity for IgG at 40 mg/mL DBC	CIP Stability	Maximum operating pressure limit	pH range
9.0 mL	≥ 40	Loading: 18.0 Operational: 27.0	360	See CIP section	4 bar (0.4 MPa)	2–13

*Dynamic binding capacity is determined using 1.25 mg/mL human polyclonal IgG in phosphate-buffered solution at 10% breakthrough with a residence time of 30 seconds. In addition, the Devices were tested with the flow restrictor off-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 Device 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 ≥ 40 mg/mL using 1.25 mg/mL human polyclonal IgG in phosphate-buffered solution at a residence time of 30 seconds, thus allowing for higher flow rates than traditional purification technologies.

LC System Recommendations

It is recommended to physically remove the flow restrictor or turn it to off-line in the software. It is recommended to replace the waste line with 3/16" diameter tubing. For ideal conditions, it is recommended to plumb system with 1 mm ID tan PEEK tubing.

Buffer And Solutions

All liquid chromatography solutions including but not limited to water, buffers, and chemical 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.

A maximum pressure setting of 0.5 MPa (5 bar) is recommended to avoid potential damage to the Device. The user should refer to the Product Safety Sheet for specific handling procedures.

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.

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	Step
Phosphate- Buffered Solution	50 mM Phosphate 150 mM NaCl	7.4 ± 0.10	Column equilibration Binding equilibration
Phosphate- Buffered High Salt Wash Solution	50 mM Phosphate 1.15M NaCl	7.4 ± 0.20	Removal of non-specific protein-protein interactions
Citrate Buffer	100 mM Citrate	3.4 ± 0.20	Elution buffer
Sodium Hydroxide	0.2 N NaOH	13.3 ± 0.40	Clean-in-Place solution
Sodium Hydroxide	0.1 N NaOH	13.0 ± 0.10	Sanitization solution
Citric acid	100 mM Citric acid	2.0 ± 0.10	Acid strip
DI Water	H_20	N/A	Used for all buffers and aqueous solutions
Ethanol / DI Water Solution	20%/80% (v/v)	N/A	Buffer for long-term storage of the protein affinity Device

DI = deionized; mM = millimolar; N = Normal; N/A = not applicable;
NaCl = sodium chloride; NaOH = sodium hydroxide; v/v = volume/volume

Sample preparation

The pH of the harvest 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 harvest stream should be filtered through a 0.2 µm filter to reduce debris and particulates.

If the harvest is prone to aggregation, it is highly recommended to perform depth filtration for further clarification of the harvest.

The purity of the incoming sample will influence Device performance and purity level of the eluted antibody.

Running Conditions

Note: Gore Devices are shipped in 20% ethanol / 80% deonized water. The Device should be rinsed with deionized water before initial use. 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. The recommended flow rate for CIP steps is 10.8 mL/min (50 seconds

residence time). A sanitization should be performed every 25 cycles using 0.1 N NaOH (at a recommended flowrate of 1.35 mL/min) for 15 minutes total contact time.

Note: Flow rates for the purification steps provide a residence time of 20 seconds (27 mL/min) and 30 seconds (18 mL/min) for a 9.0 mL Device. Furthermore, a longer residence time may increase binding capacity. The user can refer to the datasheet (PB9523) for comparison data at various residence times.

Note: A pre-conditioning run should be performed including a CIP step after long term storage (≥ 1 month) and may be run for a new Device.

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, buffer composition and pH, residence time, washing protocol, and/or elution protocol.

1. Remove the GORE Protein Capture 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.

2. Wash with 45.0 mL at a flow rate of 9.0 mL/min.
3. Equilibrate the Device with five column volumes of phosphate-buffered solution.
4. Load the Device with the harvest containing the target antibody.

   Note: Gore verified the continued performance of the Device for 100 purification/cleaning cycles by using clarified and filtered extracts harvested from CHO cells.

5. Load the sample according to the 10% breakthrough determined from an overload cycle performed prior to purification.
6. Wash the Device with six column volumes of high salt phosphate-buffered solution to remove non-specific bound material in the upflow direction (direction is optional).
7. Wash the Device with six column volumes of phosphate-buffered solution to remove salt and additional non-specific 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 harvest stream.

8. 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.

9. Strip the Device with four column volumes of 100 mM citric acid, to remove additional foulants.
10. Wash the Device with four column volumes of phosphate-buffered solution to return to neutral pH.

11. Perform CIP/sanitization by running 0.2 N sodium hydroxide for 3 minutes of contact time at a flow rate at 10.8 mL/minute in the upflow direction (direction is optional).

    Note: The customer can refer to the Clean-In-Place and Sanitization section for additional information.

12. Wash the GORE Protein Capture Device with eight column volumes of phosphate-buffered solution in the upflow direction (direction is optional) to equilibrate to a neutral pH.
13. Repeat for additional cycles starting with step 2 above; otherwise proceed to step 14 for storage.
14. Rinse the Device with five column volumes of deionized water to remove salt from the Device.
15. Add five column volumes of 20% ethanol (v/v) in deionized water to the Device using the flow rate of 9.0 mL/min.

Table 3 shows column volumes used per purification step for the 9.0 mL Device at the recommended flow rates.

Table 3. Example of an Antibody Purification Procedure

#	Step	Solution/Buffer	Column Volumes	Residence Time (seconds)	Flow Rate (mL/min)	Optional Flow
1	Equilibration	Phosphate-Buffered Solution	5	20	27	Downflow
2	Load	Harvest	Determined by sample titer	30	18	Downflow
3	High Salt Wash	Phosphate-Buffered Solution + 1 M NaCl	6	20	27	Upflow
4	Equilibration	Phosphate-Buffered Solution	6	20	27	Downflow
5	Elution	Citrate Buffer (pH 3.4)	Collection of elution peak[1]	20	27	Downflow
6	Acid Strip	100mM Citric Acid (pH 2.0)	4	30	18	Downflow
7	Equilibration	Phosphate-Buffered Solution	4	20	27	Downflow
8	CIP	0.2 N Sodium Hydroxide	3.6	50	10.8	Upflow
9	Equilibration	Phosphate-Buffered Solution	8	20	27	Upflow

[1] Collection was started at an absorbance of 100 mAU and continued until the absorbance returned to 100 mAU

Clean-In-Place and Sanitization

A CIP cycle should be performed after each purification cycle using 0.2N NaOH. The CIP should be run for 3 minutes contact time at reduced flow of 10.8 mL/min. It is recommended to perform a sanitization with 0.1 N NaOH for 15 minutes contact time at 10mL/min every 25 cycles, before switching to a new harvest or purification protocol, or on the last run of the column prior to long-term storage. For alternative NaOH concentrations see Gore application note PB10206.

Storage

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

Shelf Life

Based on accelerated aging, the Device has a shelf life of at least one year (12 months) at 2–8°C (35– 46°F) under recommended storage conditions.

Labeling

Each GORE 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 serial lot number. Each Device package is labeled with product name, part number, lot number, serial lot number, manufacture date, and expiration date.

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
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 100 cycles) is ≤20 ppm. Additional polishing may be required. • The initial cycle may have elevated Protein A levels as typical for all affinity devices, a conditioning or blank run may be performed if necessary. • Can be antibody dependent and may require additional optimization of the purification protocol to meet specific leaching requirements
HCP/other impurities in elution pool	• Alter wash buffer or solution in high salt step • Perform the high salt wash or other wash steps in the reverse direction
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

Ordering information

Part Number	Description	Quantity
PROA103	9.0 mL Device	1/each

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