GORE® Protein Capture Device
58 mL through 1 L

Operating Instructions

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Together, improving life

Product Description

The GORE Protein Capture Device - 58 mL (Figure 1a), 116 mL, 232 mL (Figure 1b), 250 mL (Figure 1c), 500 mL and 1 L (Figure 1d) are 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 polytetrafluorethylene (ePTFE) membrane composite that provides a binding capacity advantage at high flow rates (Table 1) with low delta column pressure over 100 cycles. This combination improves the speed of purification.

Figure 1a. GORE Protein Capture Device – 58 mL

Figure 1b. GORE Protein Capture Device – 116/232 mL

Figure 1c. GORE Protein Capture Device – 250 mL

Figure 1d. GORE Protein Capture Device – 500 mL and 1 L

Intended Use

GORE Protein Capture Devices, 58 mL, 116 mL, 232 mL, 250 mL, 500 mL, and 1 L are intended for clinical applications and process development.

Device Characteristics

Table 1. GORE Protein Capture Device Characteristics

Nominal Bed Volume	$DBC_{10\%}$* (g/L)	Residence Time (seconds)	Common Flow Rates (mL/min)	Capacity for IgG at 40 g/L $DBC_{10\%}$ at 30 seconds residence time loading (g)*	Clean in Place (CIP) Stability	Pressure	pH range
58 mL	≥ 40	30	116	2.32	See CIP section	Typical operating pressure < 4 bar (0.4 MPa) Burst Pressure > 8 bar (0.8 MPa)	2–13
		20	174				
		10	348				
116 mL		30	232	4.64			
		20	348				
		10	696				
232 mL		30	464	9.28			
		20	696				
		10	1392				
250 mL		30	500	10			
		20	750				
		10	1500				
500 mL		30	1000	20			
		20	1500				
		10	3000				
1 L		30	2000	40			
		20	3000				
		10	6000				

*Dynamic binding capacity is determined using 3 g/L 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

Operate the GORE Protein Capture Device (58, 116, 232 mL, and 250 mL) with flow running in the direction specified by the flow arrow on the label. If pressure develops over time, reverse the flow direction and reduce flow to 20 mL/min for cleaning. Refer to *Reverse flow cycles at higher flow rates with the GORE Protein Capture Device with Protein A* technical note (PB11997).

The 500 mL and 1 L have both the inlet and outlet on the top. The outlet is designated with the word outlet. The inlet is inline with the integrated air trap

Flow Rate Selection

The GORE Devices have a dynamic binding capacity (DBC) of ≥ 40 g/L using 3 g/L human polyclonal IgG in phosphate-buffered solution at a residence time of 30 seconds, thus allowing for higher flow rates than traditional purification technologies. It is recommended to use residence time (RT) when scaling rather than linear velocity. Calculating flow rate for a given residence time is as follows:

$$\text{Flow rate (mL/min)} = \frac{\text{(device volume (mL))}}{\text{RT (seconds)} \times \frac{\text{1 minute}}{\text{60 seconds}}}$$

LC System Recommendations

It is recommended to use an AKTA Pilot or similar sized LC system due to recommended flow rates and desired pressure for the 58 mL, 116 mL, 232 mL and 250 mL sizes. If looking to operate the 232 mL or 250 mL at 10 seconds residence time (1.4 L/min) or faster use a system similar to an AKTA Process or AKTA Ready.

It is recommended to use an AKTA Process or AKTA Ready or similar sized LC system due to recommended flow rates and desired pressure for the 500 mL and 1 L sizes.

The size of the thread for the 58 mL is 5/16-20 flat bottomed threaded fitting. The 116 mL, 232 mL, 500 mL and 1 L have a 3/4” tri-clamp (0,984” OD) fitting. The 250mL has a 1/2-20 threaded connector and should use 1/4” tubing if on the AKTA Pilot.

The 58 mL device can be used on system similar to an AKTA 150 if the following adjustments are made: physically remove the flow restrictor or turn it to off-line in the software; replace the column inlet and outlet tubing with 3/16” tubing; and 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.

Disposal After Use

Dispose of this column and/or its contents according to local, state, national, and international regulations.

Please refer to the product safety sheet (PB10387) for more information.

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.

The device was validated to not leak or burst up to 0.4 MPa (4 bar).

Refer to the Product Safety Sheet (PB10387) for specific handling procedures.

The 58 mL device is designed with 5/16-24 flat-bottom 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 250 mL is designed with 1/2-20 flat bottom 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 116 mL, 232 mL, 500 mL, and 1 L devices are designed with 3/4" Tri clamp (0.984" (OD)) connections.

Air Removal

The GORE Devices should have no air as shipped; however, there may be instances where a small amount of air is introduced during handling or connecting to the system. A small air bubble will not cause issues or disruption of the column performance. In order to remove air from a 58 mL, 116 mL, 232 mL, 250 mL, 500 mL, or 1 L device,

a) Orient 58 mL, 116 mL, 232 mL, or 250 mL device vertically with the outlet at the bottom and the inlet at the top. The 500 mL or 1 L device should be oriented vertically with the inlet and outlet at the top. Ensure that the column tubing is free of air then remove the device fitting at the inlet side.

b) Set a flow rate of 0.5 mL/min of water or the desired solution in down flow and connect the inlet column tubing to the inlet of the device. The outlet device fitting should still be sealed. Pause the system flow.

c) Without moving the device, remove the fitting from the outlet side and connect the outlet column tubing to the outlet connector of the device.

d) Resume flow of up to 60 SRT through the device in down flow for approximately 2 column volumes.

e) Reverse the flow direction. Flow in up flow until all air has evacuated the dome on the integrated air trap.

Note: If bubbles are present in the 58 mL or 250 mL they should be visible at the top of the inlet as designed. The integrated air trap (IAT) on the 116 mL, 232 mL, 500 mL, and 1 L should trap the bubbles readily and be visible in the dome of the IAT.

e) Once all air bubbles are removed from both the device and the column tubing, return to down flow and equilibrate the device with the desired solution.

f) Ensure the device is held as level as possible during the air removal and testing. It is recommended to fully transition the device at 20 SRT.

Note: In reverse flow, operating at flow rates greater than those associated with 30 seconds residence time is not recommended.

Example of 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.

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% / 80% (v/v) ethanol / deionized water. Rinse device with deionized water or equilibration buffer 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.

Clean-In-Place and Sanitization

Perform a CIP with each purification cycle using 0.2N NaOH. Run the CIP for 3 minutes contact time at a reduced flow of 50 SRT.

A sanitization may be run with 0.1N NaOH at 3 minutes residence time (MRT) for 5 CV every 25 cycles, before switching to a new harvest or purification protocol, or on the last run of the column prior to storage.

If higher molarity is desired, see application note PB10206 for methodology to estimate total contact time.

Example Purification Protocol

The recommended residence time for the purification steps are 20 SRT or 30 SRT. A longer residence time may increase binding capacity. Refer to the datasheet (PB10133) for comparison data at various residence times. The operational steps may be performed as fast as 15 seconds residence time for loading and 7 seconds residence time for non-loading (demonstrated in *GORE® Protein Capture Devices: Increased Productivity at Multiple Scales with Rapid CIP* Application Note PB12180), however it is recommended to observe pressure to be within preferred delta column pressure of 0.4 MPa (4 bar).

The column volumes for each step should be optimized for buffers used. The column volume ranges in Table 2 provide ranges tested by Gore with a select monoclonal antibody. The system hold-up volume may be used in the determination of total column volumes needed per step.

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.

Any external air trap or bubble trap should be bypassed when using Gore Devices as it will add unnecessary hold-up volume. Introduction of air will not disturb the membrane bed and the design of the 58mL and 250 mL and integrated air trap in the 116 mL, 232 mL, 500 mL, and 1 L should capture air at the inlet.

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.

Initial Set-up

1. Assuming air removal and initial set-up has been performed as indicated previously, equilibrate the GORE Protein Capture Device with deionized water or equilibration solution using at least five column volumes, at 1 minute residence time. Flow may gradually be increased by monitoring pressure.

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

2. Equilibrate the Device with five to ten column volumes of equilibration solution.

Cycling Protocol

1. 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.

2. Load the sample according to desired binding capacity at appropriate residence time.

   Note: At the end of the load step, an optional step of 1-2 column volumes of equilibration solution may be run at loading residence time to flush any harvest onto column as a result of system hold-up volume.

3. Wash the Device with four to six column volumes of a wash solution such as a high salt buffered solution to remove non-specific bound material.

   Note: This step may be optional and starting with existing platform solutions is recommended to determine if additional optimization is necessary.

4. Wash the Device with four to six column volumes of 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.

5. Elute the antibody from the Device with four to six column volumes of elution buffer using a 100% isocratic elution. Eluted fractions should be neutralized with preferred buffer.

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

6. Strip the Device with four column volumes of low pH acid, approximately pH 2, such as 100 mM citric acid, to remove additional foulants.

7. *Optional:* Wash the Device with four column volumes of buffered solution to return to neutral pH.

8. Perform CIP/sanitization by running 0.2 N sodium hydroxide for 3 minutes of contact time.

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

   An alternative rapid CIP step may be performed by running 0.5 N sodium hydroxide through the column for 30 seconds of contact time, as demonstrated in Application Note PB12180.

9. Wash the GORE Protein Capture Device with 4-10 CV of buffered solution to equilibrate pH and conductivity before loading more of the target antibody.

10. Repeat for additional cycles starting with step 2 above; otherwise proceed to step 11 for storage.

11. Rinse the Device with 4-10 CV of water to equilibrate pH and conductivity before loading more of the target antibody.

12. Add five column volumes of 20% / 80% (v/v) ethanol / deionized water to the Device using a reduced flow rate of 30-60 SRT to prevent over-pressurization of the Device.

Table 2 shows column volumes used per purification step as a sample protocol.

Table 2. Example of an Antibody Purification Cycling Procedure

#	Step	Solution/Buffer	Column Volumes	Residence Time (seconds)
1	Load	Harvest	Determined by sample titer	30
2	Equilibration	Phosphate-Buffered Solution	4 - 6	10 - 20
3	High Salt Wash	Phosphate-Buffered Solution + 1 M NaCl	4 - 6	10 - 20
4	Equilibration	Phosphate-Buffered Solution	4 - 6	10 - 20
5	Elution	Citrate Buffer (pH 3.4)	4 - 6	10 - 20
6	Acid Strip	100mM Citric Acid (pH 2.0)	4	10 - 20
7	Equilibration	Phosphate-Buffered Solution	4	10 - 20
8	CIP	0.2 N Sodium Hydroxide	3.6	10 - 20
9	Equilibration	Phosphate-Buffered Solution	4 - 10 as needed	10 - 20

Storage

Store the GORE Protein Capture Device in 20% / 80% (v/v) ethanol / deionized water at a temperature range of 2–8°C (35– 46°F). After storage perform a blank run with CIP step. Before and after storing the device for an extended period of time, perform a sanitization with 0.1N NaOH at 3 MRT for a volume of 5 CV.

Shelf Life

Based on accelerated aging, the devices have 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 and 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 • Include an acid strip after elution
Leakage at fittings	• Tighten the 5/16-24 or 1/2-20 fittings and ensure proper alignment with the thread (58 mL or 250 mL, respectively) or ensure gasket and TC clamp are aligned and tight (for 116 mL, 232 mL, 500 mL, and 1 L).
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 0.1 N 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 and harvest 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 either with high salt wash or pH steps, this will be influenced by harvest and molecule.
Elution peak tailing	• If possible, lower pH of the elution buffer • If possible, increase the ionic strength of the elution buffer
Air bubbles introduced	• Ensure solutions and LC tubing lines are free of air bubbles • Ensure the Device isn't tilted during flow • Check equipment filters and sensors for air or clogging • Any air bubbles captured during operation will be visibly trapped at the top of the Device. To remove air captured during operation: - Stop system flow - Reverse the flow direction. Flow in reverse flow at 60 SRT until all air has evacuated the top of the Device. Reverse flow can be performed as fast as 30 SRT, demonstrated in Technical Note PB11997. - Stop system flow. Return to forward flow direction, and resume operation.
Device was dropped	• Check the inlet and outlet connector to ensure no damage by testing with appropriate threaded fitting or TC connector. • Survey device for any cracks prior to use • Verify no cracks by connecting device to LC and testing with a low flow rate to assess for leaks, cracks or damage

Ordering information

Part Number	Description	Quantity
PROA201	58 mL Device	1/each
PROA202	116 mL Device	1/each
PROA203	232 mL Device	1/each
PROA301	250 mL Device	1/each
PROA302	500 mL Device	1/each
PROA303	1 L Device	1/each

NOT INTENDED FOR USE in medical device or food contact applications or with radiation sterilization.

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