



## AcroSep™ SDR Columns

- Pre-packed columns for detergent removal.
- High dynamic binding capacity for small hydrophobic molecules.
- Versatile use:
  - Manually in combination with a syringe
  - Fully automated in combination with an automated chromatography instrument such as the ÄKTAdesign\* systems
  - Semi-automated in combination with pumps

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### Ordering Information

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<b>Part Number</b>	<b>Description</b>	<b>Color Code</b>	<b>Column Volume (CV)</b>	<b>Packaging</b>
20033-C001	SDR HyperD®	Natural	1 mL	5/pkg

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**Note:** *The procedures herein are intended only as a guide. Users should always verify product performance with their specific applications under actual use conditions. If you have questions about the information presented in this guide, please contact Pall Life Sciences technical service.*

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## Specifications

### Materials of Construction

Column Housing, Cap, Plug, and Adapter: Polypropylene

Column Frit: Polyethylene

### SDR HyperD® Properties

Particle Structure: Porous silica bead

Particle Size: 40-100  $\mu\text{m}$

Polymer: Hydrophobic long chain aliphatic

Exclusion Limit: 10 kDa

Specific Capture Surface Area: 200  $\text{m}^2/\text{g}$

Binding Capacity for Triton<sup>®</sup> X-100:  $\geq 90 \text{ mg/mL}^\dagger$

Operation pH Range: 2-12

Volume Changes Due to pH and Ionic Strength: None, non-compressible

### Column Geometry

Column Volume: 1.04 mL

Bed Height: 1.48 cm (0.58 in.)

Bed Diameter: 0.94 cm (0.37 in.)

### Device Dimensions

Diameter: 1.6 cm (0.6 in.)

Length (Without Plugs): 4.8 cm (1.9 in.)

### Connections

Inlet: Threaded female luer

Outlet: Rotating male luer locking hub

### Flow Rates

Recommended for automated systems: 1-4 mL/min

### Maximum Column Pressure

Maximum: 3 bar (300 kPa, 43.5 psi)

### Storage Conditions

2-30 °C; do not freeze

<sup>†</sup>Determined using 5 mg/mL Triton X-100 in PBS, pH 7.2; 10% breakthrough, 300 cm/hr.; 1.66 mL sorbent, Column ID 4.66 mm, Column length 100 mm



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## Specifications (continued)

### Chemical Composition and Structure

SDR HyperD® chromatography separations rely on mixed mode interactions, specifically a combination of hydrophobic and size exclusion. Molecules that bind must be small, due to the 10 kDa exclusion limit, and hydrophobic to interact with the long chain aliphatic group. The mixed mode nature of this resin is created by combining a porous, spherical, silica bead with a hydrophobic polymer. The polymer is uniformly distributed throughout the silica pores allowing the specific interaction of small molecules in solution with silanol and hydrophobic groups. Molecules greater than 10 kDa are excluded from the beads. The polymer is highly cross-linked to prevent any ligand leaching during use.

### Capacity

The binding capacity of SDR HyperD resin depends on:

- The target molecule
- The residence time of the sample on the column (flow rate)
- The characteristics of the biological liquid

### Detergent Depletion Example (Data generated in an AcroSep™ Chromatography Column)

[Sample in PBS, pH 7.2; Flow rate 3.5 mL/min (300 cm/hr)]

Sample	Triton* X-100 DBC†	BSA DBC
Triton X-100 @ 5 mg/mL	≥ 90 mg/mL	-
Triton X-100 + BSA @ 5 mg/mL	≥ 90 mg/mL	< 1 mg/mL
BSA @ 5 mg/mL	-	< 1 mg/mL

†Dynamic Binding Capacity (DBC)

Albumin does not bind to the SDR HyperD resin. Triton X-100 binding capacity is unchanged in the presence of 5 mg/mL BSA.

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## **Specifications (continued)**

### **Chemical Stability**

SDR HyperD® chromatography media is insoluble in water and in organic solvents. It is also very stable to strong denaturing agents, detergents, and chaotropic agents. Its stability in acidic aqueous solutions is exceptionally high. As a result, classical chemical treatments (except strong alkaline solutions) for pyrogen removal can be performed without changing the properties of SDR HyperD sorbent, see **Pre-use Sanitization** section below.

### **General Adsorption Conditions**

- Equilibrate the column in running buffer (e.g., PBS) at intended flow rate, until the pH, ionic strength, and UV baselines are stable.
- Inject the sample containing the molecule to be removed (e.g. Triton® X-100) into the column.
- Wash with running buffer.

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### **Pre-use Sanitization**

Before use it may be desirable to sanitize or remove pyrogens from the resin. The following procedure is recommended.

- Wash with at least 3 CV of a solution of 20% (v/v) ethanol containing 1 M acetic acid.
- This solution should be injected at a flow rate of 10-20 cm/hr.
- Recommended contact time is 1 hr.
- After treatment, re-equilibrate with normal, sterile, pyrogen-free buffer.
- Do not use NaOH for sanitization.



## Instructions for Use – Manual Use with Syringe

### Materials Required

- Syringes with luer lock fittings
- Filtered buffers

### Syringe Protocol

**Note:** *It is important to avoid introducing air into the column. Remove air bubbles from fluid filled syringe before attachment to the column each time the syringe is changed. When pushing fluid through the syringe, maintain a relatively constant flow rate with minimal backpressure, typically 1-4 mL/min.*

1. Fill the syringe with buffer. To avoid getting air into the column, fill syringe with more than the required amount of buffer.
2. Equilibrate the column with 5-10 CV of buffer by securing the filled syringe to the column luer connector. Check that there are no air bubbles at the site of attachment then apply gentle pressure to push the buffer through the device.
3. Fill the syringe with sample containing detergent.
4. Attach the syringe to the column luer connector. Check that there are no air bubbles at the site of attachment then apply gentle pressure to push the sample through the column.
5. Collect flow through sample. This is the detergent-depleted sample.
6. Wash with at least 5 CV of running buffer to ensure that any remaining sample is removed from the column.

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## **Instructions for Use – Automated or Pumped Chromatography Systems**

### **Materials Required**

- System (ÄKTAdesign\* System, pump, or equivalent)
- Filtered buffers

### **Automated System Protocol**

1. Attach column to pre-primed system. To prevent air from getting into the column, fill the neck of the column dropwise while system is running very slowly. Allow the buffer to flow through the column until all bubbles in the bottom of the column have been evacuated.
2. Equilibrate with 5-10 CV of running buffer.
3. Load the sample containing detergent to be removed.
4. Collect flow through sample. This is the detergent depleted sample.
5. Wash with at least 5 CV of running buffer to ensure that any remaining sample is removed from the column.



## Determination of Binding Capacity Using an Automated Chromatography System

### Adsorption Conditions

- Equilibrate the column in PBS buffer using the working flow rate until the pH, ionic strength, and UV baselines are stable.

### System Parameters

- Intended flow rate: 1-4 mL/min.
- Equilibration: 10 CV buffer.
- Sample load: Load sufficient quantity of sample to exceed the capacity. Volume is dependant on binding affinity, concentration, and conditions. Choose these to match expected conditions for actual sample run. Pall Characterization Conditions, 70 mL injection of 5 mg/mL of Triton\* X-100 in PBS, pH 7.2.
- Void volume ( $V_0$ ): To determine  $V_0$ , perform a run in the bypass position (sample does not go through the column).

### Calculation

- DBC Formula:

$$DBC = C \times (V_L - V_0)$$

C = Concentration of load

$V_L$  = Volume at 10% or 50% breakthrough

$V_0$  = Total volume passing through the system from the time of injection (for Triton X-100, 0% deflection of  $OD_{280}$ ) until detergent breakthrough (for Triton X-100, increase in  $OD_{280}$ ).

**Note:** *Same protocol can be used for binding capacity determination for other detergents. Other detergents may require alternate means of detection.*

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## Adapter Recommendations

AcroSep™ pre-packed columns are made with a luer inlet and outlet for easy connection to syringes. The following table lists recommendations if adapters are needed to connect the columns to other types of tubing.

<b>Connection To</b>	<b>Adapters (Upchurch Scientific*)</b>
1/16" OD PTFE and Tefzel* Tubing	1 kit (P-837) <i>Instructions provided with kit</i>
1/8" OD PTFE and Tefzel Tubing	1 kit (P-838) <i>Instructions provided with kit</i>
1/16" Stainless Steel Tubing	1 inlet fitting (P-658), 1 outlet fitting (P-655), 2 ferrules (P-259), 2 nuts (LT-115) <i>Instructions provided with fittings</i>
1/32" Stainless Steel Tubing	1 inlet fitting (P-658), 1 outlet fitting (P-655), 2 ferrules (P-248), 2 nuts (LT-115) <i>Instructions provided with fittings</i>

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#### **WARNING**

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