Instructions for Use

SDR HyperD®

Solvent-Detergent Removal Resin

1 Product Description

Solvent-Detergent Removal (SDR) HyperD® resin is made of silica beads in which the pore volume is filled with a three-dimensional cross-linked hydrophobic polymer.

The particle size distribution (40 – 100 µm), the small pore size of the silica beads and the hydrophobic nature of the chemical groups make SDR HyperD® resin an excellent tool for specific solvent-detergent removal from biological liquids, after a virus inactivation step, for instance.

The silica moiety confers a good efficiency in detergent capture and a high degree of rigidity, which allows the resin to withstand high pressures. Its microporosity prevents non-specific protein capture from the biological liquid. The polymer moiety provides hydrophobicity for an efficient solvent capture (e.g. Tri-n-Butyl Phosphate — or TnBP). The resulting polymeric network confers an excellent chemical stability in both aqueous and organic solvents, while non specific sorptions are dramatically minimized.

SDR HyperD® resin is available suspended in 20% ethanol as bacteriostatic.

2 Properties

Particle size	40 – 100 µm (av.)
Exclusion limit	10 kDa
Binding capacity for Triton™ X-100*	≥ 90 mg/mL*
Operating pH range	2 – 12
Volume changes due to pH and ionic strength	Non compressible
Pressure resistance	70 barg (1,000 psig)
Usual working pressure	< 3 barg (44psig)

* Determined using 5 mg/mL Triton™ X-100* in PBS, pH 7.4, 10% breakthrough, 300 cm/h.

2.1 Main Benefits

— Microporous, rigid and hydrophobic resin.
— High mass transfer (good resolution and dynamic capacity) for small molecules due to HyperD® resin.
— Low non-specific adsorptions.
— Mechanical stability.
— Chemical stability in acid and polar organic solutions.
— Physical stability at high temperature.
— Sterilizable with oxidizing agents.

2.2 Chemical Composition and Structure

SDR HyperD® is a rigid resin in spherical bead form constituted of a network of silica and hydrophobic polymer. The particle size is 40 – 100 µm.

The polymer is uniformly distributed in any empty space of the microporous silica skeleton, allowing the specific interaction of solvent-detergent with silanol and hydrophobic groups on the matrix contrary to proteins or any macromolecule for steric hindrance reasons. The polymer is highly cross-linked, to prevent any leakage during separation operation and cleaning steps.

2.3 Porosity

SDR HyperD® resin has a microporous structure (10 KDa exclusion limit) and offers the following advantages:

— Sieving phenomena avoiding non-specific trapping of proteins or others macromolecules.
— High specific area (200 m²/g) providing high solvent-detergent dynamic capacity.

2.4 Capacity

The solvent-detergent sorption capacity of SDR HyperD® resin depends on:

— The residence time of the sample on the column,
— The flow rate,
— The characteristics of the biological liquid.

Sorption capacities for bovine plasma are:

— Triton™ X-100*: 60 – 80 mg/mL
— TnBP: 40 – 50 mg/mL

Table 1: Resin-Detergent Depletion Example

		Before Depletion	After Depletion	Removal Efficiency
IgG	TnBP	5 mg/mL	< 0.4 ppm	< 99.9%
	Triton™ X-100*	10 mg/mL	< 10 ppm	< 99.5%
ATIII	TnBP	5 mg/mL	< 0.4 ppm	< 99.9%
	Triton™ X-100*	10 mg/mL	< 10 ppm	< 99.5%
Bovine serum	TnBP	5 mg/mL	< 0.4 ppm	< 99.9%
	Triton™ X-100*	10 mg/mL	< 10 ppm	< 99.5%

Sample volume: 3.6 cv, Flow rate: 150 cm/h; Column length: 10 cm (3.9 in.); Residence time: 4 min

2.5 Mechanical Stability

SDR HyperD® resin is non-compressible.

2.6 Chemical Stability

SDR HyperD® resin 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.

SDR HyperD® packings can be washed repeatedly with 0.01 to 0.1 N hydrochloric acid or water-miscible organic solvent without undergoing substantial modification of the general properties of the matrix. This means that classical chemical treatments (except strong alkaline solution, for silica degradation reasons) for cleaning or pyrogen removal can be performed without changing the properties of SDR HyperD® resin. For more drastic cleanings, see specific section below.

2.7 Thermal Stability

Based on silica and chemically cross-linked polymer, SDR HyperD® resin is stable over a wide range of temperatures. It can be autoclaved (20 min at 121°C | 250°F).

3 Column Packing

3.1 Packing a Small column (<= 5cm I.D.)

SDR HyperD® is compatible with traditional low or medium pressure chromatography columns and equipment.

For preliminary laboratory studies, a column equipped with an adjustable piston, of 1 cm I.D. x 20 cm length, facilitates optimal packing. Direct scale-up is accomplished by maintaining bed height constant while diameter is increased. We recommend a chromatography system able to deliver a minimum linear flow rate of 300 cm/h for packing. 600 cm/h will be recommended when a pressure versus flow rate curve is required.

3.1.1 Preparing the Resin Slurry

⚠ **Do not use stainless steel paddles or magnetic stirrers as they may damage the beads and create fine particles.**

1. Gently agitate the container to fully suspend the resin.
2. Depending on the desired bed volume, transfer a suitable volume of slurry to a graduated beaker including a "practical" excess of resin (20 to 25% of the desired bed volume are recommended).
3. Allow the resin to settle and remove the supernatant. Add 3 to 5 volumes of packing buffer (20% ethanol) be used.
4. Gently agitate the slurry and let the resin settle. Remove the supernatant and add 3 to 5 volumes of fresh packing buffer.
5. Repeat step 4, three times minimum in total.
6. Gently agitate the slurry, pour it into a measuring cylinder and allow the resin to settle.
7. Remove the supernatant and add a volume of packing buffer equal to one-half the volume of settled resin. A slurry of 67% (v/v) — the concentration recommended for packing — is obtained. Alternatively, the slurry concentration can be estimated by centrifuging between 20-g and 200-g about 10 mL of homogenized slurry in a graduated vial tube for 5 minutes. The concentration of slurry expressed as: [resin volume | total volume (resin + supernatant) x 100] can be directly estimated in the tube. The 10 mL slurry can be poured back into the total suspension before packing.

3.1.2 Preparing Column and System for Packing

1. Prime the solvent delivery system, lines and valves to assure that all air is displaced. Assure that top and bottom frits or nets are fully wetted and free of air. Fill the column with packing buffer and operate the system over a range of flow rates representative of values that will be used during packing and anticipated chromatographic procedures.
   Record pressure associated with the empty column and system (including detectors, etc...).
2. Determine pressure | flow characteristics for the empty system, taking into account recommendations concerning flow rate during packing and chromatography operation.
3. Stop the pump, close the column outlet, and remove the upper flow adaptor or piston.
4. Open the outlet and drain buffer from the column, leaving 1 – 4 cm of buffer above the bottom frit. Following completion of blank pressure | flow measurements, proceed with column packing as described below.

3.1.3 Packing the Column

1. Gently resuspend the slurry and pour it into the column in one continuous motion against the wall of the glass tube to minimize introduction of air bubbles (if the pouring process is done in several motions, gently homogenize the slurry in the column using a plastic rod).
2. Fill with packing buffer (20% ethanol) up to the top of the glass tube. Allow the suspension to settle so that a layer of clear supernatant ≤ 1 cm is visible at the top of the column.
3. Connect the upper piston to the system and prime with packing buffer to ensure that no air is trapped under the net or frit. Stop the pump and insert the adjustable piston into the column. Tighten it.
4. Open the column outlet and operate the pump at a selected linear velocity of 300 cm/h (~3.9 mL/min for a 1 cm I.D. column) for efficient packing.
5. When the top of the bed stabilizes, stop the pump and untighten the piston. Position the adjustable piston at the top of the packed resin, leaving no visible space between the frit and packed resin at any point around the circumference.
6. Operate the pump again and repeat the adjustment of the piston until no visible space appears under flow (300 cm/h) between the frit and the top bed.

4 Working Conditions and Basic Protocol

4.1 Column

For a first use, a column length of 15 cm (5.9 in.) minimum and a working flow rate between 100 and 300 cm/h are recommended.

4.2 Adsorption Conditions

— Equilibrate the column in PBS buffer using the working flow rate, until the pH, ionic strength and UV baselines are stable.
— Inject 5 to 10 cv of the clean sample containing the solvent-mixture to be removed (e.g. 1% Triton™ X-100* and 0.5% TnBP) into the column, followed by the starting buffer.

4.3 Washing Conditions

The most commonly used procedure to desorb solvent-detergent molecules from the hydrophobic resin is to inject 1 cv of PBS / EtOH 95° (50/50) followed by 3 cv of EtOH 95°. If necessary, 2-isopropanol can also be used. According to the sample composition, 10 cv of PBS / EtOH 95° (50/50) can be used, followed by 10 cv of EtOH 95° and 10 cv of 2-isopropanol.

5 Cleaning

After packing and | or between runs, it may be necessary to sterilize and eliminate pyrogens from the column. This may be performed as follows:

Method	Procedure
Alcohol	acid treatment	Wash with at least 3 cv of a solution of 20% (v/v) ethanol containing 1 M acetic acid. This solution should be injected after removal of dissolved gas at a flow rate of 10–20 cm/h (1 hour contact time). After treatment, reequilibrate with normal sterile pyrogen-free buffer.

6 Thermal Stability and Storage

Temperature of use	2 – 30 °C
Storage temperature	2 – 30 °C (2 – 8 °C once used)
Storage solution	Neutral buffer containing a bacteriostatic agent such as 20% (v/v) ethanol
⚠	Product must never be frozen
i	Product is shipped at ambient temperature

7 Ordering Information

Pack Size	Part Number
25 mL	20033-031
100 mL	20033-023
1 L	20033-015
5 L	20033-056
10 L	20033-049

For more information, please contact our technical service.

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