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User Guide

Agilent PLRP-S Bulk Media Packing

General description

Agilent PLRP-S media is a wide-pore polymeric polystyrene/divinylbenzene particle with inherent hydrophobicity, making it suitable for reversed-phase chromatography. It is supplied as a dry powder.

PLRP-S bulk media comes in different particle and pore sizes:

- 8, 10, 10–15, 15–20, 30, 50 µm
- 100, 300, 1000, 4000 Å

Bulk media is provided in 10 g, 100 g, or 1 kg quantities. For specific part numbers, please see the ordering information table at the end of this guide.

The operational instructions contained in the user guide supplied with the column and packing station must always be adhered to.

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Preparation of the slurry and column packing

Note: Read the column and packing station user manual for a comprehensive guide to packing a PLRP-S preparative column.

1. Calculate the amount of media required to pack the column. The amount required is an empty column volume multiplied by the optimum packed bed density.
   a. To calculate the empty column volume, multiply $\pi r^2 h$ (where r = radius in cm, or half of the column diameter, and h = bed height/length of the column in cm), or reference the calculator in this link.
   b. The optimum packed bed density for PLRP-S depends on pore size. See Table 1 for the optimum packed bed densities.

Table 1. Values of packed bed densities for Agilent PLRP-S bulk media.

PLRP-S Bulk Media Pore and Particle Size	Packed Bed Densities (g/mL)
PLRP-S 100 Å, 8 µm, 10 µm, 10-15 µm, 15-20 µm, 50 µm	0.27
PLRP-S 300 Å, 8 µm, 10 µm, 10-15 µm, 15-20 µm, 50 µm	0.27
PLRP-S 1000 Å, 10 µm, 30 µm, 50 µm	0.27
PLRP-S 4000 Å, 10 µm, 30 µm	0.24

Example

Calculating the amount of PLRP-S 1000 Å, 10 µm media for an Agilent Load & Lock 4001 column with a desired bed height of 150 mm: 27 × 150 mm (2.7 × 15 cm):

Empty column volume: $\pi r^2 h$. $\pi(2.7/2)^2 \times 15 = 85.9$ mL

The amount of media is the column volume multiplied by the packed bed density:

85.9 mL × 0.27 g/mL = 23.2 g of PLRP-S 1000 Å required

2. PLRP-S media is supplied as a dry powder. Using the necessary precautions to avoid inhalation of particles, carefully weigh the required amount of media that was calculated in step 1.
3. Prepare sufficient packing solvent to create a slurry concentration of approximately 0.2 g/mL. An appropriate packing solvent is 80:20 v:v acetonitrile:water.

Example

Calculating the amount of mobile phase needed to make 0.2 g/mL:

0.2 g/mL = 23.2 g/x mL
x = 23.2/0.2 = 116 mL

4. Disperse the particles before dispensing; this is best done using a bottle roller or through gentle shaking. **Do not use a magnetic stirrer or a stirrer that can grind the particles against the sides or bottom of the vessel.** Once the media is fully dispersed, ensure there are no lumps. If necessary, pass the slurry through a coarse sieve to remove any lumps. Do not attempt to force lumps through the sieve as this could damage the particles. The slurry is now ready for column loading and may be used immediately or stored for up to one month. If stored, the PLRP-S material will settle and must be fully dispersed using a bottle roller or gentle shaking before column packing.
5. Take the homogeneous, free-flowing PLRP-S slurry and pour it smoothly into the assembled column.
6. Complete the assembly of the column (Figure 1) according to the manufacturer's instructions.

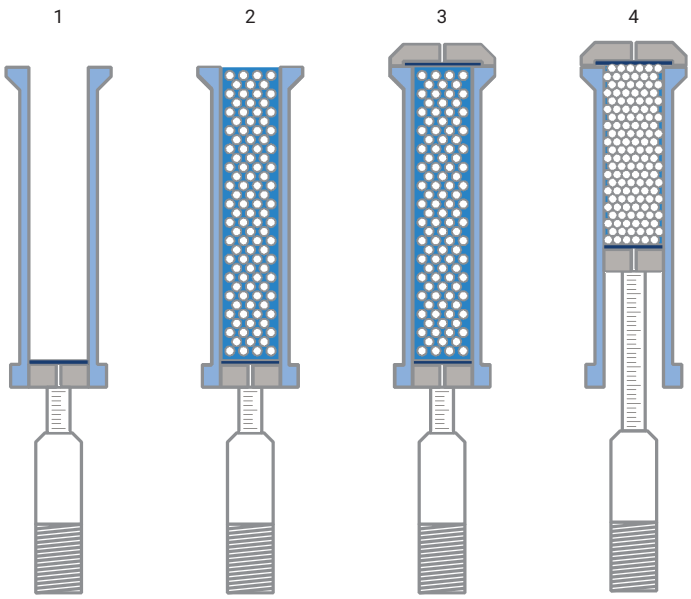

Figure 1. A diagram demonstrating the steps to pack Agilent PLRP-S. (1) Assemble the column on the packing rig. (2) Pour the slurry into the empty column. (3) Assemble the column end fitting. (4) Use the hydraulic pump to load the column.

7. To load the column using dynamic axial compression, it is important not to overpressurize the media, as this will compress the particles, reducing column permeability, and resulting in a higher column operating pressure. It is recommended that the pressure applied to the chromatographic bed be low, and the piston pressure be set to low and gradually increased so that the media is not overpressured. Carefully observe the graduated hydraulic ram and pack the column until the desired stationary phase bed height is reached. Do not overpack or compress the particles any further.
8. The compression ratio for the hydraulic pressure to the bed pressure for the Load & Lock columns vary with size. The recommended bed compression pressure is approximately 650 psi, which translates to different hydraulic pressures based on compression ratios. See Table 2 for values. If a non-Load & Lock system is being used, please see the manufacturing instructions.

Table 2. A summary of recommended hydraulic pressures, based on Agilent Load & Lock column dimensions. Packing pressures may vary by particle size; 10 µm particles are usually packed at ~ 10% higher pressures than 30 µm particles.

Column	Compression Ratio for Hydraulic:Bed Pressure	Recommended Bed Compression Pressure (psi)	Hydraulic Pressure (psi)
Load & Lock 4001, 27 × 500 mm	1:2.5	650	260
Load & Lock 4002, 50 × 500 mm	1.5:1	650	975
Load & Lock 4003, 75 × 500 mm	3:1	650	1,950

9. A piston pressure of approximately 650 psi (45 bar) is likely to be the maximum pressure needed for Load & Lock columns. Make sure that the packing pump pressure has been calculated using the correct ratio for the column id/packing station to give a piston pressure of 650 psi.
10. Once the column loading is complete, the mobile phase flow has stopped, and the bed length has been achieved, allow the column to equilibrate for 15 minutes.
11. Lock the column piston into position so that the column can be operated in static axial compression mode.
12. Do not continue to apply pressure to the piston, as this can cause particle compression, particle damage, reduced permeability, and high pressures.
13. The column is now ready for use.
14. Connect to the HPLC pump. Starting at a low flow rate (about one-quarter of the method flow rate), gradually increase until it is at the rate that will be used for the purification. Then, flush the column with high-strength mobile phase, followed by low-strength mobile phase. Finally, connect the column outlet to the detector and continue flushing until a stable baseline is achieved.
15. It is recommended that, before applying sample, a blank gradient is run to ensure that the column/system is operating correctly.

Column testing

Once the column has been equilibrated, it can be tested for efficiency, retention time, and symmetry. The column should be flushed and tested with a suitable test probe that does not interact with the media. A suitable test probe is acetone tested using 80:20 v:v acetonitrile:water as mobile phase. If necessary, the acetone can be diluted in the mobile phase.

The column test should be performed at the flow that will be used for the purification.

Table 3. Suggested flow rates for various column dimensions.

Inner Diameter	Flow Rate (mL/min)
4.6 mm	0.5 to 1.0 (180 to 360 cm/h)
27 mm (Load & Lock 4001)	15 to 30
50 mm (Load & Lock 4002)	60 to 120
75 mm (Load & Lock 4003)	130 to 260

Note: Column efficiency and pressure are dependent on the system configuration, including tubing length and id, and detector cell volume.

Ordering information

PLRP-S	10 g	100 g	1 kg
8 µm 100 Å	N/A	N/A	PL1412-6800
10 µm 100 Å	PL1412-2100	PL1412-4100	PL1412-6100
10–15 µm 100 Å	PL1412-2400	PL1412-4400	PL1412-6400
15–20 µm 100 Å	PL1412-2200	PL1412-4200	N/A
50 µm 100 Å	PL1412-2K00	PL1412-4K00	PL1412-6K00
8 µm 300 Å	N/A	PL1412-4801	PL1412-6801
10 µm 300 Å	PL1412-2101	PL1412-4101	PL1412-6101
10–15 µm 300 Å	PL1412-2401	PL1412-4401	PL1412-6401
15–20 µm 300 Å	PL1412-2201	PL1412-4201	PL1412-6201
50 µm 300 Å	PL1412-2K01	PL1412-4K01	PL1412-6K01
10 µm 1000 Å	PL1412-2102	PL1412-4102	PL1412-6102
30 µm 1000 Å	PL1412-2702	PL1412-4702	PL1412-6702
50 µm 1000 Å	PL1412-2K02	PL1412-4K02	PL1412-6K02
10 µm 4000 Å	PL1412-2103	PL1412-4103	PL1412-6103
30 µm 4000 Å	PL1412-2703	PL1412-4703	PL1412-6703

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