thermo scientific PRODUCT INFORMATION SHEET

Packing and Testing Conditions for POROS™ Self Pack™ Technology 20 A Media

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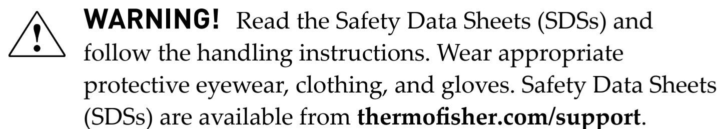

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Read this before you read the packing device document

Use this instruction sheet in conjunction with the document titled *POROS™ Self Pack™ Packing Device for High Performance Perfusion Chromatography™ Columns* (shipped with the packing device).

Note: "Packing the column" on page 2 of this instruction sheet replaces "Packing the column" in the *POROS™ Self Pack™ Packing Device Product Information Sheet*.

Begin by reading the *POROS™ Self Pack™ Packing Device Product Information Sheet*. When you reach Section 4.3, "Packing the Column", switch to "Packing the column" on page 2 of this instruction sheet.

Instructions at the end of "Packing the column" on page 2 direct you back to the appropriate section of the *POROS™ Self Pack™ Packing Device Product Information Sheet*.

Contents of this instruction sheet

For the media you have purchased, this instruction sheet tells you:

- The specific slurry conditions, packing conditions, and packing instructions for Perfusion Chromatography™ systems (BioCad™ Workstation or BioCAD Sprint™ System) or FPLC™ systems.
- The specific slurry conditions, all packing conditions except flow rate, and packing instructions for other HPLC systems.
- The expected performance of a properly packed column.

Preparing the slurry

Note: POROS™ 20 A media is packaged in slurry form, using 20% ethanol as a preservative. This slurry is not ready for use in the packing device. You must prepare the slurry before using it in the packing device.

This section tells you what solvents to use and how to slurry the media.

Solvents

WARNING! Always wear eye protection when working with solvents.

Use these solvents:

- Slurry solvent (to slurry the media): 100% ethanol
- Packing solvent: 100% ethanol

Slurrying the media

The bottle of POROS™ media slurry contains enough slurry, when prepared, to pack the following number of columns:

Table 1 Number of columns

Media Quantity	Number of Columns	
	4.6 mmD/50 mmL	4.6 mmD/100 mmL
(0.8 g)	2	1
(2.7 g)	6	3

To prepare the media:

1. Swirl the media bottle gently until the slurry is well suspended.
2. Pour the slurry into a beaker:
   - For a 4.6 mmD/ 50 mmL column: 3 ml
   - For a 4.6 mmD/100 mmL column: 6 ml
3. Add slurry solvent to the beaker:
   - For a 4.6 mmD/ 50 mmL column: 10 ml
   - For a 4.6 mmD/100 mmL column: 7 ml
4. Stir the slurry until the additional solvent is evenly distributed.
5. Store any slurry remaining in the original media bottle at 4°C.

Packing the column

Note: This section replaces "Packing the Column" in the *POROS™ Self Pack™ Packing Device Product Information Sheet*.

Note: Set system pressure before you pack your column. See "Preparing Your LC System" in the *POROS™ Self Pack™ Packing Device Product Information Sheet* for information.

If you are packing a column using a Perfusion Chromatography™ system or a conventional HPLC system, install a backpressure regulator on your column. See "Using the Backpressure Regulator" in the *POROS™ Self Pack™ Packing Device Product Information Sheet* for information.

Packing conditions

For all systems and column sizes:

1. Add13 ml of prepared slurry to the device.
2. Top the device off with slurry solvent as described in "Packing the column" on page 2 in this instruction sheet.

Principles of packing

As you pack a column with POROS™ 20 A media, the backpressure rises as the water in the slurry solvent is pushed from the column and falls after the water in the slurry solvent is out of the column.

The optimal packing flow rate is the highest flow rate possible that allows packing to proceed within the pressure limit you set in "Preparing Your LC System" in the *POROS™ Self Pack™ Packing Device Product Information Sheet*.

The maximum packing flow rate is dependent on the system used, the particle size of the media, and the length of the column.

To keep within the pressure specifications of FPLC™ pumps, program your FPLC™ system to deliver the flow rate with pump A and B (set "Conc % B" to **50** and prime both pumps with packing solvent).

Packing a column using the BioCad™ Workstation, BioCad™ Sprint™ System, or FPLC™ System

Note: Record the highest sustained pressure that occurs during packing. Do not exceed this pressure when you run the packed column.

To pack a column:

1. Set the flow rate:
   - For Perfusion Chromatography™ systems: 14 ml/min
   - For FPLC™ systems: 6 ml/min

   These flow rates apply to both 4.6 mmD/50 mmL and 4.6 mmD/100 mmL columns.
2. Turn on the flow.
3. Monitor the system pressure and the volume of packing solvent in the waste beaker below the column.

   The backpressure rises as solvent passes through the column. After about 25 ml of solvent has passed through the column, the pressure drops. This pressure rise and drop occurs as the slurry solvent is pushed out of the column. You can now increase the flow rate for the rest of the packing process.
4. Increase the flow rate:

   Note: The packing flow rate has been determined to allow you to comfortably pack your column within the pressure limit of your system. However, variations in frit permeability or system backpressure may require you to adjust the flow rate slightly.
   - For Perfusion Chromatography™ systems, both column sizes: 20 ml/min
   - For FPLC™ systems:
     - 4.6 mmD/50 mmL columns: 10 ml/min
     - 4.6 mmD/100 mmL columns: 8 ml/min
5. Run another 25 ml of packing solvent through the column.

   If your system is well below the pressure limit during packing, increase the flow rate in 0.5 ml/min increments until you approach the pressure limit. Pack columns at the highest flow rate possible without exceeding the system pressure limit.

   If you reach the pressure limit before you finish packing the column, stop the flow, restart the flow at 0.5 ml/min lower than the original setting, and continue packing. Repeat if necessary until you are able to pack the column within the pressure limit.

   Note: Occasionally a defective frit generates excessive backpressure. If so, replace the frits and start again.
6. Stop the flow.
7. Let the column sit for 3 minutes to allow the pressure to dissipate evenly across the packed bed.
8. Record the highest sustained pressure that occurred during packing.

   Do not exceed this pressure when you run the packed column.
9. Immediately flush the column with 10 column volumes of 50 mM phosphate pH 7.0/0.15 M NaCl to remove ethanol from the column. Extended exposure to high organics can denature the protein A.
10. Go to "Removing and Capping the Packed Column" in the *POROS™ Self Pack™ Packing Device Product Information Sheet*.

Packing a column using other systems

Note: Monitor the highest sustained pressure that occurs as you pack the column. Do not exceed this pressure when you run the packed column.

If you are using systems other than the BioCad™ Workstation, BioCad™ Sprint™ System, or FPLC™, you need to experiment to find the correct flow rate. Start by setting the flow rate at the maximum allowable on the system, but not higher than 14 ml/min.

To pack a column:

1. Turn on the flow and monitor the volume of packing solvent coming through the column by observing the level in the waste beaker below the column.

   The pressure rises as solvent passes through the column. After about 25 ml of solvent has passed through the column, the pressure drops. This pressure rise and drop occurs as the slurry solvent is pushed out of the column and the system. You can now increase the flow rate for the rest of the packing process.

2. Run another 25 ml of packing solvent through the column.

   If you reach the pressure limit before the required volume of packing solvent passes through the column, stop the flow, restart the flow rate at 0.5 ml/min lower than the original setting, and continue packing.

   If the pressure limit is again exceeded, lower the flow rate another 0.5 ml/min.

   Continue lowering the flow rate in 0.5 ml/min increments until the necessary volume of packing solvent passes through the column.

   Note: Occasionally a defective frit generates excessive backpressure. If so, replace the frits and start again.

3. Stop the flow.

4. Let the column sit for 3 minutes to allow the pressure to dissipate evenly across the packed bed.

5. Record the final flow rate. Use this flow rate the next time you pack a column with your system.

6. Record the highest sustained pressure that occurred during packing.

   Do not exceed this pressure when you run the packed column.

7. Immediately flush the column with 10 column volumes of 50 mM phosphate pH 7.0/0.15 M NaCl to remove ethanol from the column. Extended exposure to high organics can denature the protein A.

8. Go to "Removing and Capping the Packed Column" in the *POROS™ Self Pack™ Packing Device Product Information Sheet*.

Packed column performance

This section describes:

- The maximum recommended flow rate for your packed column
- How to do a permeability test on your packed column
- The conditions necessary to run a protein separation test on your packed column

You can verify your success each time you pack a fresh column and monitor column performance over time. See "Testing the Column" in the *POROS™ Self Pack™ Packing Device Product Information Sheet* for information about periodic testing.

Recommended maximum flow rate

The maximum recommended flow rate for the column during normal operation is 85% of the packing flow rate. This flow rate keeps the pressure within the operating limit you recorded in “Packing the column“ on page 2 in this document.

When you work with viscous solvents, lower the operating flow rate to account for the greater pressures generated by the greater viscosity.

Permeability

Column pressure/flow characteristics are called column *permeability.* Test column permeability at the recommended maximum flow rate to establish a baseline.

To test column permeability:

1. Run 50 mM phosphate pH 7.0/0.15 M NaCl buffer through the column at the recommended maximum flow rate.

2. Record the generated pressure (permeability baseline).

Whenever you re-test column permeability, do so under solvent and flow rate conditions identical to those of this initial test.

Protein separation

Before you run the protein separation test, equilibrate the column with 10 to 15 column volumes of buffer (see Table 2 on page 3). Column bed volumes are:

- For a 4.6 mmD/ 50 mmL column: 0.8 ml
- For a 4.6 mmD/100 mmL column: 1.66 ml

Then run the protein test standard under these conditions:

Table 2 Protocol

These conditions are common to all column sizes	
Buffer	50 mM phosphate pH 7.0/0.15 M NaCl
Eluent	0.1% (v/v) (12 mM) HCl/0.15 M NaCl
Flow rate	1800 cm/hr (5 ml/min)
Sample	20 µl
Detection	280 nm

To prepare the protein test standard:

1. Dissolve the test standard in 1 ml of buffer (concentration—5 mg/ml bovine serum albumin, 5 mg/ml human gamma globulins).

WARNING! All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.

2. Filter the test standard using a 0.22 µ filter.

3. Store unused reconstituted test mix frozen.

Results

Compare your chromatogram with Figure 1 on page 4 (generated on a 4.6 mmD/100 mmL column packed with a Perfusion Chromatography™ media). To verify that your column is operating properly, confirm that the two proteins on your chromatogram are separated and the peaks are symmetrical. The areas of the two peaks may change according to the purity of the proteins in the standard, but variation in area is not important in measuring the resolving power of the column. Retention times and bandspreading on your separation may be different but the general profile should be similar.

If the general profile is not similar, make sure that your system is functioning properly. If your system is functioning properly, unpack your column and start again (see "Unpacking a Column" in the *POROS™ Self Pack™ Packing Device Product Information Sheet*).

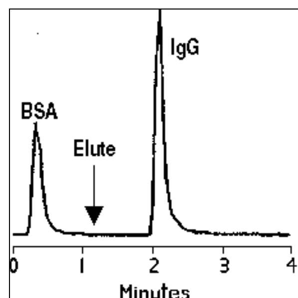

Figure 1 POROS™ 20 A chromatogram

Support

For service and technical support, go to **thermofisher.com/poros** or call toll-free in US: 1.800.831.6844.

For the latest service and support information at all locations, or to obtain Certificates of Analysis or Safety Data Sheets (SDSs; also known as MSDSs), go to **thermofisher.com/support**, or contact you local Thermo Fisher Scientific representative.

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Revision	Date	Description
B	18 January 2017	Baseline for this revision history.

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