POROS™ AL, EP, and OH Perfusion Chromatography™ Columns for Activated Affinity Chromatography

Pub. No. 8-0005-40-1093 **Rev.** C

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**.

Note: These instructions cover the specific operational characteristics of POROS™ prepacked columns. POROS™ media are also available in bulk quantities for direct scale-up of separations developed on prepacked columns. Contact your Thermo Fisher Scientific representative for more details.

Read this section before you begin

Your new POROS™ column is unique

Thermo Scientific™ POROS™ columns are made for Perfusion Chromatography™ flow-through particle chromatography—a patented new technology that performs bioseparations 10 to 100 times faster than conventional HPLC or LC without loss in capacity or resolution.

Although POROS™ columns can be operated on standard HPLC instrumentation, they are substantially different from any columns you have used before. You may have to change the way you run, and, to some extent, the way you think about chromatography. In particular, the higher flow rates made possible by Perfusion Chromatography™ allow you to perform experiments you might once have considered a luxury, given the constraints of conventional chromatography's longer run times.

Please read the operating instructions carefully to ensure that you run the column to its full capability and take maximum advantage of the benefits that Perfusion Chromatography™ technology provides.

Increase the flow rate

The largest single difference between POROS™ columns and conventional columns is the flow rates under which high capacity and resolution are achieved.

Although the column can be operated at flow rates typical of conventional chromatography, the full benefits of Perfusion Chromatography™ can be realized only by increasing the flow rate so that the linear velocity is in the range of at least 1,000 to 5,000 cm/hr. Higher flow rates are possible but may not be practical, depending on the system you are using, the viscosity of your solvents, or the pressure rating on the column.

Linear velocity (cm/hr) is calculated by dividing volumetric flow rate (cm^3/min) by the column cross-sectional area (cm^2) and multiplying by 60 min/hr.

Note: If you operate with a peristaltic pump, you may need to run at lower flow rates to keep within the pressure rating of the pump.

The typical volumetric flow rate ranges for various column diameters are provided in Table 1 on page 1:

Table 1 Typical flow rates for Perfusion Chromatography™

Column Diameter (mm)	Flow Rate Range (ml/min)	Linear Velocity (cm/hr)
2.1	0.5–5	900–8,500
4.6	3–15	1,000–5,300
10	15–75	1,100–5,700
16	30–150	900–4,500

The dramatically higher flow rates of POROS™ columns and media introduce new considerations into the design and execution of experiments. This is particularly true when you adapt a method developed on conventional media.

Be sure to read "Guidelines for using Perfusion Chromatography™" on page 5 for a full discussion of these considerations.

Reoptimize your method as needed

You may need to reoptimize the separation to account for possible differences in selectivity between the POROS™ column and your old column.

The short run times associated with Perfusion Chromatography™ make optimization quick and easy, especially if you are using the BioCad™ Workstation for Perfusion Chromatography™.

Product description

POROS™ AL, EP, and OH columns are polymeric packings designed for affinity chromatography of pipettes, proteins, polynucleotides, and other biomolecules with user-immobilized legends in the Perfusion Chromatography™ mode. The packings consist of cross-linked poly(styrene-divinylbenzene) flow-through particles with a patented bimodal, pore-size distribution for rapid mass transport. This base matrix is coated with a cross-linked polyhydroxylated polymer (POROS™ OH), which is then activated with aldehyde (POROS™ AL), or epoxide (POROS™ EP) functional groups.

POROS™ OH is designed to be activated by the user, using any of the conventional chemistries such as glutaraldehyde, tresyl, divinylsulfate, CNBr, and so on.

POROS™ AL, EP and OH column packages include the following items:

- Packed column with sealing end caps
- *Product Information Sheet*
- Column Test Certificate
- Fitting Adaptor Kit (P-Series columns only)
- EZ™ Grip stainless steel fittings

POROS™ columns series and sizes

POROS™ activated affinity columns are available in different performance series and sizes. Check that the column you purchased is appropriate for your application and is compatible with the pressure limits of the system you use (see Table 2 on page 2). Refer to "Guidelines for using Perfusion Chromatography™" on page 5 for a detailed discussion of pressure considerations for running Perfusion Chromatography™ columns on conventional systems.

Table 2 Classification of POROS™ activated affinity columns

Particle Size	Series	Maximum Pressure Drop	Type of Separation	Chromatography System
20 µm	M	170 bar (2,500 psi, 17 MPa)	Analytical or Preparative	BioCad™ Workstation, or conventional HPLC instrumentation
20 µm	P	30 bar (450 psi, 3 MPa)	Analytical or Preparative	BioCad™ Workstation, medium pressure FPLC™-type instruments, or low-pressure peristaltic pumps

Table 3 Product characteristics

Support Matrix	Cross-linked poly(styrene-divinylbenzene)	
Surface Functionality	POROS™ OH	Hydroxyl
	POROS™ AL	Aldehyde
	POROS™ EP	Epoxide
Shipping Solvent	0.1 M Na_2SO_4, 30% methanol	
Packing Density	0.35 g/ml	
Shrinkage/Swelling	<1% from 1–100% solvent	

Table 4 Chemical resistance

pH Range	POROS™ AL, OH POROS™ EP	1–14 (up to 5.0 M NaOH or 1.0 M HCL) 3–9
Ionic Strength Range	0–5 M, all common salts	
Buffer Additives	All common agents, including 8 M urea, 6 M guanidine hydrochloride, ethylene glycol, and detergents. **WARNING! CHEMICAL HAZARD. Guanidine hydrochloride** may be harmful if swallowed or absorbed through the skin. Exposure may cause eye, skin, and respiratory tract irritation and adverse effects on the central nervous system and bone marrow. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **WARNING!** Do not expose to strong oxidizers (such as hypocrite), oxidizing acids (such as nitric acid), or strong reducing agents (such as sulfate).	
Solvents	Water, 0–100% alcohols, acetonitrile, other common organic solvents. **WARNING! CHEMICAL HAZARD. Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, central nervous system depression, and damage to the heart, blood system, liver, and kidneys. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.	
Operating Temperature	5–80 °C	

Connecting the column

The column fitting is a Parker 10-32 female fitting.

POROS™ columns come with E-Z Grip™ stainless steel fittings that are designed to be tightened by hand.

IMPORTANT! With PEEK columns, do not use standard steel fittings that require tightening with a wrench. Overtightening can strip the column threads.

Extra E-Z Grip™ fittings are available from **thermofisher.com**. See "Accessories, spare parts, and ordering information" on page 6.

Connecting the column

P-series columns can be connected to M-6 metric fitting systems (such as the Pharmacia FPLC™ system) by using the supplied fitting adaptor kit provided.

1. Connect the short tubing section to the column using the red Fingertight fittings.
2. Slip the black metric nuts over the other end of the tubing, followed by a blue ferrule, with the conical end *pointing toward the nut*.
3. Connect the nut to a female M-6 fitting.

The columns can also be connected to 1/4-28 fitting systems using the green 1/4-28 male nut in place of the black M-6 nut.

General considerations

General considerations, regardless of chemistry, are provided below. Guidelines (and in some cases, suggested protocols) for specific surface chemistries are provided in the following sections.

In activated affinity chromatography, coupling yield (ratio of molecule offered to molecule immobilized), surface concentration (mass of the immobilized molecule per unit support column bed volume), and specific activity (biological activity per unit mass of immobilized molecule), are determined by the following:

- Binding buffer (pH)
- Concentration of molecule in binding solution
- Solubility of the molecule in binding solution
- Ratio of molecule mass to support mass or bed volume
- Concentration of support in binding solution
- Reaction time and temperature
- Reaction quenching
- Mode of reaction (batch or column)

Because molecules for immobilization differ substantially in reactivity and other properties, the optimal conditions for immobilization can be determined only experimentally. However, published papers and textbooks should be consulted, where appropriate.

Surface concentration versus yield and activity

In general, maximum surface concentration is achieved by high concentrations of both molecule and support and high ratios of molecule to support.

However, high surface concentration is often obtained at the expense of low coupling yield and specific activity of the immobilized molecule.

Note that the surface concentration of reactive groups on POROS™ activated supports has been carefully optimized to reduce overcoupling, which causes low specific activity.

Maximum utilization of rare or expensive molecules is usually achieved at a lower ratio of molecule to support, and thus lower surface concentration.

Use of salting out conditions

In general, with macromolecules such as proteins, coupling under salting out conditions can achieve both high coupling yield and high surface concentration.

To achieve this, add a saturated solution of sodium sulfate or other anti-chaotropic salt to the coupling solution until the solution just

starts to become cloudy (just before the concentration reaches the precipitation point). Avoid ammonium sulfate because primary amines in the buffer interfere with all the reaction chemistries.

Although the support surface is quite hydrophilic, it is less hydrophilic than the solution. When exposed in high-salt concentration, the protein partially precipitates on the support surface. This dramatically raises the local concentration at the surface, driving the coupling reaction to a much higher level.

Preparation of concentrated sodium sulfate

At the high concentrations required in a salting out procedure (2 M, for example), sodium sulfate can precipitate during preparation. To prevent precipitation:

1. Dissolve the appropriate amount of solid sodium sulfate in hot (> 80 °C) water in a volume close to the final buffer volume.
2. Allow the solution to cool slowly at room temperature.
3. When the temperature of the solution reaches approximately 30 °C titrate to the final pH, using as small a volume as possible so that the buffer does not cool too rapidly.
4. Allow the buffer to cool to 25 to 30 °C.
5. Proceed with the immobilization.

Make sure all other buffers are at ambient temperature.

If prepared carefully, 2 M sodium sulfate is stable at room temperature.

Batch reactions

Batch reactions are generally more flexible than columns for immobilization development, because the concentrations of ligand molecule and support can be readily varied independently.

An Activated Affinity Development Kit containing 10-ml samples of each of the activated chemistries is available for use in batch reactions. Refer to "Accessories, spare parts, and ordering information" on page 6 for details.

Preparing the column

POROS™ activated affinity columns are shipped in 0.1 M sodium sulfate (Na_2SO_4), 30% methanol.

Before you use the column for the first time, pump the column with 5 to 10 column bed volumes of water to remove the methanol.

Before adding the molecule to be immobilized, pump the column with at least 10 column bed volumes of the coupling buffer.

Coupling in a packed column can be easy and quick, especially when optimal reaction conditions have already been worked out. Regardless of the coupling chemistry you use, always do the following:

- Prepare the column for the reaction by washing extensively (at least 10 column bed volumes) with the coupling buffer.
- Form the binding solution by dissolving or exchanging the molecule into the coupling buffer. For proteins, the concentration should typically be between 10 and 20 mg/ml. Lower concentrations give higher coupling yield; higher concentrations give higher surface concentration.
- If your system is set up to recirculate binding solution, use a minimum volume at a slow flow rate.

  Alternatively, pump in small aliquots of binding solution until you observe breakthrough.

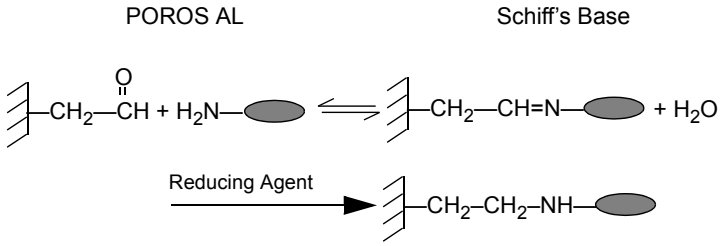
  If you have excess molecule, you can also make up the solution in a large enough volume to allow recirculation with a pump during the coupling reaction. This method works well when excess molecule is available.
- If binding solution is not recirculated, after the column is filled with binding solution, remove the column from the system, close both ends, and store it at the desired reaction temperature for the desired time.
- Do not leave the system in coupling buffer, particularly if coupling involves salting out. Wash the system extensively with water.
- After immobilization and quenching reactions are complete, wash the column extensively with coupling buffer.
- Prepare the column for affinity chromatography by washing with 5 to 10 column volumes of elution buffer, followed by 10 to 15 column volumes of starting/wash buffer.

POROS™ AL

Chemistry

The figure below shows the reaction chemistry of POROS™ AL media.

- Aldehyde groups on the surface of the support react selectively with primary amines on the molecule, ultimately to form a Schiff's base.
- The Schiff's base is subject to easy hydrolysis. Therefore, a highly stable secondary amine linkage is formed by reacting the Schiff's base with a mild reducing agent.



Coupling buffer

When you select a coupling buffer:

- Do not use any buffer that contains primary amines, such as Tris.
- The optimum coupling pH is usually between 4 and 8.
- pH 6 to 7 usually produces good results.
- Salting out conditions are recommended. See "Use of salting out conditions" on page 2 for details.

Reaction time and temperature

The required reaction time depends on the ligand and the reaction temperature.

Reaction times vary:

- At room temperature, allow at least 8 hours.
- At cold-room temperature, allow up to several days.

Generally, the longer the initial reaction time, the higher the surface concentration and coupling yield.

When in doubt, perform an overnight reaction at room temperature for good results, especially with proteins.

IMPORTANT! Do not use cold-room temperature if salting out conditions have been used. Salt crystals that form may damage POROS™ media.

Reduction step

After the initial reaction and Schiff's base formation, you must react the support with a reducing agent to form a stable bond.

Consider the following when selecting a reducing agent:

- Sodium cyanoborohydride ($NaCNBH_3$) is the most widely used reducing agent. It is mild enough to prevent reduction of disulfide bonds on proteins, but it is toxic and may present disposal problems when used on a large scale.
- Do not use sodium borohydride ($NaBH_4$) in prepacked columns. Although it reduces aldehyde groups, eliminating the need for a quenching reaction, it may produce gas bubbles.

To chemically reduce the support:

1. Make up your reducing agent as a concentrated solution.

   Five mg of reducing agent per ml of column bed volume is suggested.
2. Depending on the reducing agent, begin the reduction reaction as follows:
   - **Cyanoborohydride:** Simultaneously with the coupling reaction by mixing the protein solution, coupling solution, and cyanoborohydride

- **Other reducing agents:** Add the reducing agent after the coupling reaction is complete.

3. Allow the reduction reaction to proceed for approximately 2 hours at room temperature or 8 hours in a cold room.
4. After the reduction reaction is complete, wash the support with coupling buffer.

Quenching

Residual aldehyde functionality is likely to remain after the coupling reaction. To quench these aldehydes:

1. React the support with a low-molecular-weight primary amine, such as:
   - 0.2 M Tris buffer
   - 1 M ethanolamine in phosphate buffer
2. Include reducing agent in the quenching reaction mixture.

Quenching reactions are generally complete after 2 hours at any temperature.

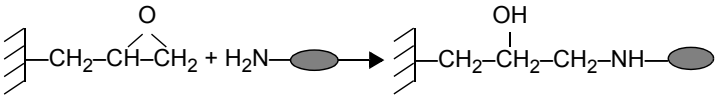
POROS™ EP

Chemistry

The following figure shows the reaction chemistry of POROS™ EP media.

- The oxirane (epoxide) rings on the support react with primary amines to form secondary amine linkages.
- The epoxide groups also react with sulfhydryl and hydroxyl groups.
- At the elevated pH used for coupling, there is a slow competing hydrolysis of the epoxide groups to form diols.

POROS EP



Coupling buffer

When you select a coupling buffer:

- Do not use any buffer that contains primary amines, such as Tris. Recommended buffers are carbonate, borate, and phosphate.
- The optimum pH for coupling is usually in the range 9 to 11.
- Salting out conditions are highly recommended. See "Use of salting out conditions" on page 2 for details.
- Organic solvents can be used if you add an appropriate organic-soluble base.
- Because of the hydrolysis reaction, POROS™ EP has a limited shelf life after coupling buffer is added.
- If the packing is to be stored in coupling buffer for any significant length of time, keep the packing cold, but DO NOT FREEZE IT.

Reaction time and temperature

The required reaction time depends on the :

- Molecule being coupled
- pH
- Reaction temperature

The reaction is faster at higher pH and temperature and with small-molecule ligands. However, the competing hydrolysis reaction is also faster. Reaction times vary as follows:

- **At room temperature with proteins** –The reaction can take place overnight.
- **At cold-room temperature** –The reaction requires several days.

IMPORTANT! Do not use cold-room temperature if salting out conditions have been used. Salt crystals that form may damage POROS™ media.

Generally, the longer the initial reaction time, the higher the surface concentration and coupling yield.

Quenching

Some residual epoxide functionality is likely to remain after the coupling reaction. To quench these epoxides, use any of these low-molecular-weight reactants:

- 0.2 M Tris buffer
- 1 M ethanolamine in phosphate buffer
- 0.1 M mercaptoethanol in phosphate buffer

Quenching reactions are generally complete after 2 hours at any temperature.

POROS™ OH

POROS™ OH media offer a surface that is chemically similar to base cellulose or agarose supports that contain a high concentration of hydroxyl functionality. Activate POROS™ OH media with any of the coupling chemistries used for agarose or cellulose matrixes, including:

- Cyanogen bromide
- Tresyl
- Glutaraldehyde
- Divinylsulfone
- Carbonyldiimidazole
- Periodate
- Triazine
- Diazonium

Consult standard textbooks for specific chemistries and protocols.

Affinity chromatography

- The binding and elution buffers used for affinity chromatography depend entirely on the nature of the ligand interaction.
- Although the POROS™ support itself and the coupling linkage are highly stable chemically, be careful not to expose the column to conditions that cause denaturation of the immobilized molecule itself.
- Because Perfusion Chromatography™ media have such a high rate of mass transport, the volumes required for complete washing and elution of the column are generally quite low (<5 column volumes).

Cleaning up and regenerating the column

POROS™ activated affinity columns are stable to most chemically resistant agents typically used in a regeneration procedure. Refer to "POROS™ columns series and sizes" on page 2 for details.

Regeneration protocol depends on the stability of the ligand and the nature of the sample containing the target molecule.

Multiple injections

You can make multiple injections of regeneration solutions instead of pumping them directly. This method is recommended for very aggressive or very viscous solvents. To clean by injections:

- Make the injection volume as large as possible.
- Use a low flow rate that allows exposure of the media to the regeneration solution for at least several minutes.

Note: Backpressure increase is sometimes caused by a plugged inlet frit. If backflushing the column does not solve the problem, replace the inlet frit. Refer to "Guidelines for using Perfusion Chromatography™ " on page 5 for details.

Storing the column

When you store your column, be sure to:

- Store the column between 5 and 30 °C.
- Store the column with the end plugs in place, carefully sealed to prevent drying. Drying results in decreased chromatographic efficiency.

Short-term storage
Store the column in any appropriate buffer.

Long-term storage
Add 0.02% sodium azide or 20% ethanol to the column.

CAUTION! Sodium azide is toxic. Follow precautions and decontamination procedures recommended by the National Institute for Occupational Safety and Health.

Guidelines for using Perfusion Chromatography™
When you make the transition to Perfusion Chromatography™, consider factors related to:

The chromatography system:

- Account for System Pressure
- Check the Gradient System
- Adjust the Data Collection System
- Maintain Your Column and System

Experimental design:

- Think in Terms of Column Volumes
- Adjust the Sample Load
- Measure Recovery Properly

Account for system pressure
The high flow rates used with Perfusion Chromatography™ cause a higher-than-usual system pressure (resulting from the chromatography hardware itself). In some cases, this system pressure can be equal to or even greater than the column pressure.

Therefore, when you use your POROS™ column, you cannot simply set the upper pressure limit of the system at the pressure rating of the column. Instead:

1. Determine the system pressure by:
   a. Connecting a union in place of the column
   b. Pumping the highest salt concentration to be used at the planned flow rate
2. Set the upper pressure limit by adding the system pressure observed in step 1 above to the column pressure rating.

If the system pressure is too high:

1. Check carefully for plugged or crimped tubing or other restrictions in your plumbing.
2. Use larger-ID or shorter tubing.
3. Use a larger detector flow cell.

In some systems, excessive system pressure can prevent the high flow rates required to take full advantage of Perfusion Chromatography™ technology.

It is important to isolate the relative contribution of the column and instrument when pressures approach the maximum column pressure. Take the column out of line to determine those contributions (measured pressure = column pressure drop + system pressure). The maximum allowable pressure drops of POROS™ columns are provided in "POROS™ columns series and sizes" on page 2.

Typical pressure drops of POROS™ columns (at 2,000 cm/hr with water as the mobile phase) are shown in Table 5 on page 5. Refer to this table to verify column performance or to help you decide if column regeneration is required.

Table 5 Typical column pressure drops

Column Series	Bar[1] (psi)		
	30 mmL	50 mmL	100 mmL
H	40 (600)	60 (900)	80 (1,200)
M	20 (300)	—	40 (600)
F	—	30 (450)	50 (750)
P	—	5 (75)	10 (150)

[1] 1 bar = 0.1 MPa

Check the gradient system
High flow rates and short run times can expose both operational and design problems in gradient blending systems. Gradient system problems can affect step changes as well as linear gradients. Most problems come from one of two sources:

- Excessive delay (dwell) or mixing volume can cause both delay in the start of the gradient at the column and rounding or distortion of the edges of the gradient. Mixing or delay volume can be reduced by using a smaller mixer and shortening the tubing between the mixer and sample injector.
- Poor gradient proportioning can cause either short-term fluctuations or long-term inaccuracies. Adding a mixer can sometimes help.

On the BioCad™ Workstation, gradient performance is tracked on each run with the internal conductivity detector. If there is any question about gradient performance on other systems, you can visualize the gradient as follows:

1. Connect a union in place of the column.
2. Form a gradient with water as the A solvent and 5% acetone in water as the B solvent with detection at 280 nm.

The UV absorbance is directly proportional to the concentration of B solvent and can be compared to the programmed gradient.

Consult your system vendor for serious gradient problems.

Adjust the data collection system
Because Perfusion Chromatography™ runs are much shorter than conventional chromatography runs, you may need to adjust your data collection system as follows:

- To obtain high-definition chromatograms, use a shorter total run time and higher data collection rate (or lower average peak width parameter). A typical data collection rate is 10 points/second.
- If you use a chart recorder, increase the chart speed in proportion to the flow rate increase.

Maintain your column and system
With Perfusion Chromatography™, you can perform runs more quickly than with other chromatography technologies. For this reason, perform maintenance tasks such as replacing filters or regenerating columns after a certain number of runs, rather than after a specified period of time. You can reduce the frequency of such maintenance by always filtering the sample and eluent.

Think in terms of column volumes
In any chromatographic separation, as flow rate increases, gradient time must decrease to maintain constant gradient volume. At the flow rates used for Perfusion Chromatography™, the gradient times are dramatically shorter than those you are accustomed to working with. To convert a method to Perfusion Chromatography™, keep the gradient volume constant in terms of column volumes and adjust the time of the gradient according to the new flow rate. Table 6 on page 5 provides bed volumes of POROS™ columns to help you make the necessary calculations.

Table 6 POROS™ prepacked columns

Diameter (mmD)	Length (mmL)	Column Bed Volume (ml)
2.1	30	0.10
2.1	100	0.35
4.6	50	0.8
4.6	100	1.7
10	100	7.9
16	100	20.1

When you work routinely with Perfusion Chromatography™, always think of gradients in terms of column volumes, because a slight change in gradient time may result in a dramatic difference in gradient volume and column performance.

For example, on a conventional 4.6 × 250 mm analytical column (volume 4.25 ml), a 45-minute run at 1 ml/min represents a 10.6 column-volume gradient.

On a POROS™ 4.6mmD/100mmL column (volume 1.7 ml), a 5 ml/min flow rate translates into 3 column volumes/min (5/1.7). Therefore, a 10.6 column-volume gradient would be completed in 3.5 minutes.

The BioCad™ Workstation allows you to program directly in column volumes.

Adjust the sample load

If the volume of your POROS™ column is different from the column you are currently using, adjust the sample volume or mass proportionally to keep the same load per unit volume of column. Refer to "Think in terms of column volumes" on page 5 for POROS™ column bed volumes to help you make the necessary calculations.

Measure recovery properly

Quantitation (recovery) measurements using peak integration are comparable run to run only if running conditions are kept nearly constant.

Flow rate affects the value of the integrated peak area for a given mass recovered, because the amount of material recovered in a peak is equal to the concentration (absorbance) times the volume. However, an integrator (or integration software) quantitates peaks by summing absorbance measurements over *time.* A change in flow rate is a change in the amount of volume over time.

Therefore, time integration does not allow comparison of recovery at different flow rates. Direct comparison of your POROS™ results with previous results on conventional chromatography may lead to the incorrect conclusion that recovery is lower on POROS™ columns.

There are several ways to compensate for limitations in time-based integration:

- Multiply the peak area by the flow rate. Unfortunately, because integrators often vary the data rate with expected peak width, this approach can give invalid results.
- Use peak height for a general comparison, but bandspreading differences due to different efficiencies or gradient slopes cause errors.
- Use peak integration at the same flow rate, but *doing this may not show important effects such as a recovery increase due to shorter time on the column with Perfusion Chromatography™.*
- Collect the peaks carefully and analyze using spectrophotometry or other methods. Make sure to develop blanks, especially when UV-absorbing components are present in the eluent.
- If peak collection is not feasible, generate standard curves by injecting increasing amounts of calibration standards of known concentration. If the measured peak area increases linearly with load, and the standard curve passes through the origin, it is very likely that recovery is high.

Accessories, spare parts, and ordering information

These following accessories are available for your POROS™ AL, EP, and OH columns:

Table 7 POROS™ AL, EP, and OH column accessories

Description		Quantity	Cat. No.
Activated Affinity Development Kit		Contains 10-ml samples of each of the activated resins	1-9515-00
Frits, PEEK	2.1mmD	Package of 5	1-9124-05
	4.6mmD	Package of 5	1-9125-05
	10mmD	Package of 1	1-9127-01
E-Z Grip™ Fittings (SS)		Package of 5	5-1011-05
Fitting Adaptor Kit[1]		N/A	1-9532-00

[1] The Fitting Adaptor Kit lets you connect POROS™ columns to M-6 (FPLC) and 1/4-28 low-pressure fitting systems.The kit includes two 10-32 fittings, two low-pressure ferrules, two M-6 nuts, two 1/4-28 nuts, and 1/16-inch OD PEEK tubing. The kit is included with all P-Series columns.

Column parts are available from Thermo Fisher Scientific on special order. Please inquire.

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.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

This page is intentionally blank.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. 8-0005-40-1093

Revision	Date	Description
C	10 January 2017	Baseline for this revision history.

Limited Use Label License No. 495: Internal Research, Quality Control, and Bioproduction Use for Columns and Resins: Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product, under intellectual property rights that are owned and/or controlled by Life Technologies and relate specifically to the product, to perform (a) internal research for the sole benefit of the purchaser; (b) quality control testing conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties; and (c) performance of research or manufacturing services conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties. The purchase of this product does not grant the purchaser any additional rights, including, without limitation, the right to transfer or resell the product in any form, the right to use the product as a therapeutic agent or diagnostics test component, or to use the product to perform tests other than what is indicated in this Limited Use Label License on a contract or fee per test basis for or on behalf of third parties. For information on obtaining additional rights, please contact **outlicensing@thermofisher.com** or Out Licensing, Life Technologies Corporation (part of Thermo Fisher Scientific), 5823 Newton Drive, Carlsbad, California 92008.

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288