POROS™ R1 and R2 Perfusion Chromatography™ Columns for Reversed-Phase Chromatography

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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**.

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, you can realize the full benefits of Perfusion Chromatography™ 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 of 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.

IMPORTANT! If you are operating with a peristaltic pump, you may need to run at lower flow rates to keep within the pressure rating of the pump.

Table 1 on page 1 provides the typical volumetric flow rate ranges for various column diameters.

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 4, for a full discussion of these considerations. Another excellent reference is The Busy Researcher's Guide to Biomolecule Chromatography, available from your Thermo Fisher Scientific Technical Representative.

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 use the VISION™ or BioCad™ Workstation for Perfusion Chromatography™.

Product Description

POROS™ R1 and R2 columns are polymeric packings designed for reversed-phase chromatography of peptides, proteins, polynucleotides, and other biomolecules in the Perfusion Chromatography™ mode. The packing consists of cross-linked poly(styrene-divinylbenzene) flow-through particles with a patented bimodal pore-size distribution for rapid mass transport.

POROS™ R1 and R2 media have somewhat different selectivity from conventional silica-based reversed-phase media. Binding strength for proteins and peptides is similar to low carbon-loading C4 supports for POROS™ R1 media and C8 supports for POROS™ R2 media. POROS™ R1 and R2 media show stronger binding toward some highly aromatic

species. In addition, the complete lack of residual silanol activity modifies the binding characteristics of ionic species.

POROS™ R1 columns are designed for very hydrophobic proteins and peptides.

POROS™ R2 columns are designed for general reversed-phase separations of proteins, peptides, and nucleic acids.

POROS™ R1 and R2 column packages include the following items:

- Packed column, with sealing end caps
- *Product Information Sheet*
- Column Test Certificate
- EZ™ Grip stainless steel fittings

POROS™ Column Sizes

POROS™ columns are available in different sizes. Check that the column you purchased is appropriate for your type of application and is compatible with the pressure limits of the system you use (see Table 2 on page 2). Refer to "Account for System Pressure" on page 4 for a detailed discussion of pressure considerations for running Perfusion Chromatography™ columns on conventional systems.

Table 2 POROS™ Reversed-Phase Columns

Particle Size	Maximum Pressure Drop	Type of Separation	Chromatography System
POROS™10 micron	170 bar (2,500 psi, 17 MPa)	Analytical	BioCad™ Workstation, or conventional HPLC instrumentation
POROS™ 20 micron	170 bar (2,500 psi, 17 MPa)	Preparative	BioCad™ Workstation, or conventional HPLC instrumentation

Table 3 Product Characteristics

Parameter	Specification
Support Matrix	Cross-linked poly(styrene-divinylbenzene)
Surface Functionality	None [native poly(styrene-divinylbenzene)]
Dynamic Binding Capacity @ 3,600 cm/hr	(lysozyme, 1% acetonitrile) POROS™ R1: 5 mg/ml POROS™ R2: 10 mg/ml
Shipping Solvent	acetonitrile in water, 7:1
Packing Density	0.30 g/ml
Shrinkage/Swelling	<1% from 1–100% solvent

Table 4 Chemical Resistance

Parameter	Specification
pH Range	1–14 (Up to 5.0 M NaOH, 1.0 M HCl)
Buffer Additives	All common agents, including THF,8 M urea, 6 M guanidine hydrochloride, ethylene glycol, and detergents.
Solvents	Water, 0–100% alcohols, acetonitrile, other common organic solvents. **WARNING!** CHEMICAL HAZARD. Acetonitrile (ACN) 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. **WARNING!** Do not expose buffer additives or solvents to strong oxidizers (such as hypochlorite), oxidizing acids (such as nitric), or strong reducing agents (such as sulfite).
Operating Temperature	5–80 °C

Connecting and Preparing the Column

The column fitting is an Upchurch Scientific™ 10-32 female fitting.

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

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

Extra EZ™ Grip fittings are available from **thermofisher.com** (see "Accessories, Spare Parts, and Ordering Information" on page 6).

Connecting the Column

Columns can be connected to M-6 metric fitting systems (such as the Pharmacia FPLC™ system) by using the fitting adaptor kit (see "Accessories, Spare Parts, and Ordering Information" on page 6).

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.

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

Preparing the Column

POROS™ R1 and R2 columns are shipped in 7:1 acetonitrile:water. Before the first injection, pump the column with at least 5 column volumes of 100% acetonitrile followed by at least 10 column volumes of starting mobile phase.

Selecting and Preparing the Mobile Phase

Regardless of the mobile phase you choose, always:

1. Use eluents of the highest purity practical.
2. Degas all eluents prior to use.

Solvents

As you select the solvent for the mobile phase, keep in mind that:

- Acetonitrile is the preferred solvent for reversed-phase chromatography.
- It is recommended that the A buffer in a water:acetonitrile gradient system contain a minimum concentration of acetonitrile (1%).
- Alcohols such as methanol or isopropanol may give poor peak symmetry or efficiency. However, adding 10% tetrahydrofuran or acetonitrile to alcohol-based mobile phases can improve chromatographic performance.

Mobile Phase

The polymeric nature of POROS™ R1 and R2 media allows them to withstand prolonged exposure to high-pH conditions. Switching to high-pH mobile phases may improve selectivity and provides new possibilities that are not available with conventional silica media. For example:

- 10 mM tribasic phosphate solution gives a pH of around 11.5 and is convenient for many applications.
- You can also use triethylamine (TEA) for high-pH mobile phases.
- Examine the effect of pH on selectivity by doing a systematic pH screening or mapping experiment at pH 2, 7, and 11.

The very high flow rates possible with Perfusion Chromatography™ allow you to perform such an experiment quickly.

Additives

The completely nonionic nature of POROS™ R1 and R2 media and the resistance to high pH allow great flexibility in the use of mobile phase additives.

- Additives such as TFA may no longer be necessary for separation performance, although they may still be needed for solubilization of the sample.
- Other additives such as hydrochloric, phosphoric, formic, or acetic acids can often be just as effective as TFA. However, adding organic acids in concentrations greater than 5 to 10% (v/v) can significantly reduce binding strength.
- Inorganic phosphate is not recommended as an additive because of poor solubility in acetonitrile.
- When sample selectivity is partially based on charge differences, adding appropriate hydrophobic additives (ion pairing agents) to the mobile phase can mimic the selectivity of silica-based media.

  Select the pH and additive so that the additive has an ionic charge opposite that of the ionic groups on the solute.
- For differences in negative charge (such as an oxidized sulfhydryl group), use agents such as 1 mM trimethyloctadecylammonium chloride at neutral or high pH.
- For differences in positive charge, use agents such as 5 mM pentane sulfonic acid or 0.1% hexafluorobutyric acid (HFBA), in place of TFA at low pH.

The effects of mobile phase solvent, pH, and additives can be determined only by experimentation. However, the very high flow rates in Perfusion Chromatography™ shorten experimentation time.

Preparing and Loading the Sample

To ensure efficient binding and prevent column plugging:

1. Dissolve or exchange samples for POROS™ R1 and R2 columns into the starting mobile phase.
2. Centrifuge or filter (0.22 or 0.45 µm) samples before injection to prevent column plugging.

   WARNING! If a sample contains more than 10 mM phosphate, other salts, or other components that may not be soluble in acetonitrile, you need to inject samples at 5% of the organic solvent concentration. Failure to do this may irreversibly foul the column.
3. Delipidate samples, if possible. Lipids can cause irreversible column fouling.

Determining the Sample Load

Many peptides and proteins have limited solubility in mobile phases that also allow binding to the column. Limited solubility may be exaggerated on the column, because the column concentrates the sample as it is injected. If the solubility limit is exceeded on the column, poor resolution or even column plugging can occur.

Because of limited solubility, the dynamic binding capacity of POROS™ R1 and R2 media is in the range of 0.1 to 5 mg/ml for most proteins.

In general, high-resolution separations are generally achieved at 20% of the total binding capacity or less.

However, the maximum loading at which a given resolution can be obtained (the loadability) depends on a number of factors, including sample solubility, column selectivity, and so on.

To perform a loadability experiment:

1. Determine the most effective elution conditions (eluent, gradient, and flow) at low loading.
2. Gradually increase the sample load (either through increasing injection volume or sample concentration or both) until the required resolution no longer occurs.
3. If you are using a VISION™ or BioCad™ Workstation, take advantage of the loading study template designed specifically for this purpose.

Note: As the loading is increased, the peaks may elute earlier on the gradient, possibly necessitating reoptimization of the gradient.

Once again, the short run times made possible by Perfusion Chromatography™ make reoptimization quick and easy, especially if you use the template features of the VISION™ or BioCad™ Workstation.

Eluting the Sample

Elution can be in either gradient or isocratic mode.

- Many peptides are optimally eluted isocratically, but because of the extremely high sensitivity to elution strength, it is usually more practical to use very shallow (<5% elution strength range) gradients.
- Gradient volumes of 10 to 20 column volumes normally provide a good compromise between resolution and peak dilution.

Note that with the very high flow rates possible with Perfusion Chromatography™, increased gradient volumes (with decreased slope and therefore increased resolution) are possible without the excessive times normally experienced with conventional media.

Cleaning Up and Regenerating the Column

- In some applications, sample molecules may not fully elute or may precipitate on the column. Regenerate the column if you observe:
- Increased bandspreading
- Loss of binding capacity
- Loss of recovery
- Increased pressure drop
- Trace or "ghost" peaks occurring during blank gradient runs

Note: In any cleanup method, reversing the flow direction is recommended to help flush out particulates and to prevent contamination of the lower part of the bed. Also, slow the flow rate to give several minutes' exposure time to the regeneration solution at each step of the cleaning protocol.

In reversed-phase chromatography, the bound species may have very limited solubility in the organic solvent required to remove them from the surface. Therefore, regeneration solutions must be both strong solubilizing agents and strong eluents. These qualities are often mutually exclusive. To manage this situation:

1. Run rapid "sawtooth" gradients from 100% of a very strong solubilizer (such as 50% acetic or phosphoric acid or 0.5 M NaOH, 1 to 3 M guanidine) to 100% of a strong eluent, (such as acetonitrile or isopropanol), and back to the solubilizer.

   Running a gradient helps achieve the correct blend of the two agents needed to remove the bound contaminant.
2. Take care to ensure that the solubilizer is miscible with the organic solvent selected. Isopropanol is a better choice with base or guanidine.

The stability of POROS™ R1 and R2 media to high pH allows for the use of harsh eluents such as 2 M NaOH for column cleaning, thereby increasing the range of regeneration options available, and extending the practical life of your column.

Multiple Injections

It is possible to use multiple injections of regeneration solutions instead of pumping them directly. This method is recommended when using 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 at least several minutes' exposure time to the regeneration solution.

IMPORTANT! 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 "Accessories, Spare Parts, and Ordering Information" on page 6 for details.

Storing the Column

When you store your column, always be sure to:

- Store the column in any appropriate mobile phase.
- Avoid long-term storage of stainless steel columns with halide (Cl) salts, because frit corrosion may result.

- 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.

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 most viscous solvent blend (approximately 30% acetonitrile) 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 Table 2.

Typical pressure drops of POROS™ columns (at 2,000 cm/hr with water as the mobile phase) are shown in Table 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

Pressure: Bar (psi) (1 = 0.1 MPa)

Column	30 mmL	50 mmL	100 mmL
POROS™ 10	40 (600)	60 (900)	80 (1,200)
POROS™ 20	20 (300)	30 (450)	40 (600)

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 are 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.

If you have any question about gradient performance, 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 0.5% 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 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 solutions you make up from solid components.

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 4 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 is completed in 3.5 minutes.

The VISION™ and BioCad™ Workstations allow 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 Table 6 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 the 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.

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 will 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.

Run the Test Standard

Run a standard to verify that your system and column are running properly. Refer to "Standard Test Protocols" on page 5 for details.

Standard Test Protocols

Use this standard test protocol for POROS™ R1 and R2 columns to troubleshoot column performance.

Chromatographic Efficiency

The Column Test Certificate, supplied with the column, indicates the chromatographic efficiency, asymmetry, and permeability of the column. The test uses a small, nonretained molecule run at low flow rate to obtain the most sensitive measurement of the packed column bed. Plate count is determined by the half-height method. For detailed test information, contact Thermo Fisher Scientific Technical Support.

Protein Separation

For the sample, use the Reversed-Phase Protein Test Standard available from **thermofisher.com**. Refer to "Accessories, Spare Parts, and Ordering Information" on page 6 for ordering information.

Run the separation with a linear gradient in acetonitrile. Run conditions are described below, including flow rate and sample load for various column sizes.

To perform the test:

1. Dissolve the lyophilized sample mixture in 1 ml of Eluent A (5 mg/ml soybean trypsin inhibitor, 5 mg/ml bovine heart Cytochrome C).
2. Filter the sample after thorough mixing.
3. Store the reconstituted test mix frozen.
4. Run the sample.

Run Conditions

Table 7 Protocol - Run Conditions Common to All Column Sizes

Factor	Run Conditions Common to All Column Sizes
Eluent A	A: 0.1% TFA in water
Eluent B	0.085%–0.1 % TFA in acetonitrile
Gradient	15–45% B in 5 minutes
Detection	220 nm

Table 8 Protocol - Flow Rate and Sample Size (Depend on Column Diameter)

Column Diameter (mmD)	2.1	4.6	10	16
Flow (ml/min)	1	5	25	50
Sample (µl)	5	20	100	200

Results

Figure 1 shows a standard chromatogram for a 4.6mmD/100mmL POROS™ 20 micron R1 column. Although the retention times and bandspreading may vary with particle size, column size, and systems, the general profile should be similar.

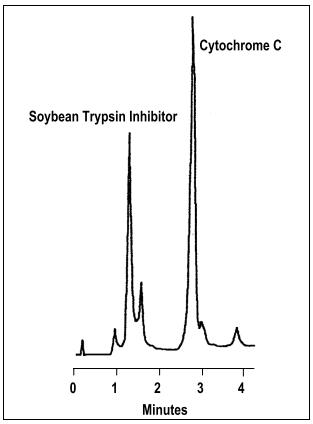

Fig. 1 POROS™ 20 Micron R1 Chromatogram

Accessories, Spare Parts, and Ordering Information

These following accessories are available for your POROS™ R1 and R2 columns.:

Table 9 POROS™ R1 and R2 Column Accessories

Description	Quantity	Part Number
Reversed-Phase Test Standards	Package of 5 vials	1-9001-05
Frits, Stainless Steel		
2.1mmD	Package of 5	1-9121-05
4.6mmD	Package of 5	1-9122-05
10mmD	Package of 5	1-9123-05
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
16mmD	Package of 1	1-9128-01
EZ™ Grip Fittings (SS)	Package of 5	P5-1011-05
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, two1/4-28 nuts, and 1/16-inch OD PEEK™ tubing.

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**.

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

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