



OPTIMIZE THE PERFORMANCE OF YOUR WATERS ALLIANCE HPLC SYSTEMS WITH AGILENT COLUMNS AND SUPPLIES

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Agilent CrossLab

From Insight to Outcome

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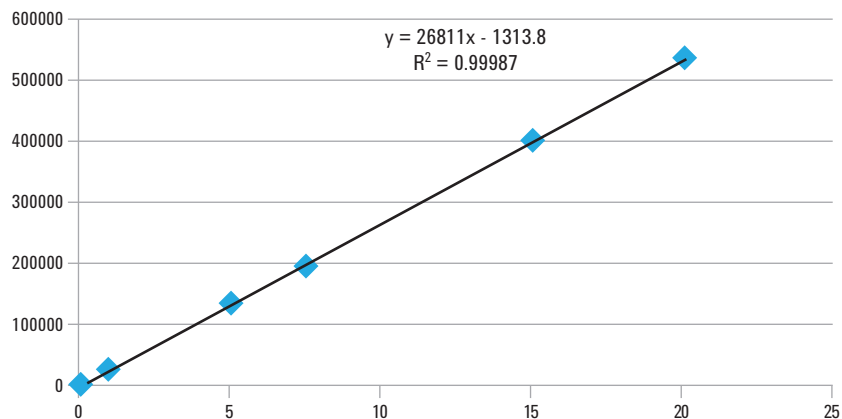
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Agilent parts work perfectly on Waters systems

To demonstrate the performance of Agilent supplies, we analyzed acetaminophen (paracetamol) on a Waters Alliance HPLC System equipped with Agilent preventative maintenance kits for the pump and autosampler, and an Agilent UV detector lamp. A Captiva syringe filter was used during sample preparation, and a Poroshell 120 EC-C18 column for the separation.

The results showed expected linearity, precision, and sample recovery values, indicating that Agilent column and syringe filter delivers reproducible and precise pharmaceutical analysis, and Agilent instrument parts for Alliance system provide excellent alternatives to Waters genuine parts.



Linear response of peak area against concentration of acetaminophen.

Accuracy, intra-, and inter-batch precision of acetaminophen analysis. The accuracy for both concentrations and all batches was well within very low error range, below $\pm 2\%$. The intra- and inter-batch RSD values were all below 0.7%, indicating significant precision.

| Concentration (µg/mL) | Batch No. | Calculated concentration (µg/mL) | Accuracy (as % error) | Intra-batch RSD (%) | Inter-batch RSD (%) |
|-----------------------|-----------|----------------------------------|-----------------------|---------------------|---------------------|
| 0.5 | 1 | 0.51 | 1.08 | 0.23 | 0.58 |
| | 2 | 0.51 | 1.41 | 0.42 | |
| | 3 | 0.50 | 0.24 | 0.19 | |
| 15 | 1 | 15.06 | 0.41 | 0.15 | 0.62 |
| | 2 | 14.85 | -1.02 | 0.11 | |
| | 3 | 14.93 | -0.48 | 0.07 | |

Read the full details of this analysis in Agilent publication 5991-6017EN.

AGILENT SUPPLIES FOR WATERS LC SYSTEMS

Detector Lamps

Our detector lamps are designed for precise alignment and thermal stability

Agilent lamps are designed and built to be compatible with a wide variety of detectors, including variable wavelength detectors (VWD), multiple wavelength detectors (MWD), and diode array detectors (DAD) or photodiode array detectors (PDA). Each lamp adheres to the tightest specifications for consistent quality and reproducible performance over the lifetime of the lamp. Test equipment is regularly calibrated using optical standards certified by NIST (National Institute of Standards and Technology) or PTB (Physikalisch-Technische Bundesanstalt).

- Manufactured in an ISO 9001 certified environment
- Quartz glass bulbs for extended lifetime
- Individually tested for light intensity, noise and drift, correct operating voltage, and proper alignment for low lamp-to-lamp variability
- Tight QA/QC with traceability for every lamp throughout every step of the product process
- Both deuterium lamps and tungsten lamps available

Deuterium lamps

- High output stability and intensity for extended detection capabilities and improved qualification at trace level
- Guaranteed lifetime of 2,000 hours

Tungsten lamps

- Offer coverage in the visible wavelength range for high sensitivity detection
- Average lifetime range from 1,200 to 5,000 hours depending on operation conditions such as operation cycles, soft start, and operation voltage

TIPS & TOOLS

Each time you replace a detector lamp, perform wavelength calibration and intensity tests after warm up.

Detector Lamps, 1/pk

| Model | Description | Similar to Waters Part No. | Part No. |
|---|---------------------------------------|-----------------------------------|-----------------|
| 2996 Photodiode Array Detector 996 Photodiode Array Detector | Long-life deuterium lamp, 2,000 hours | WAT052586 | 8005-0705 |
| 2487 Dual Wavelength Absorbance Detector 2488 Multichannel Absorbance Detector | Long-life deuterium lamp, 2,000 hours | WAS081142 | 8005-0704 |
| 486 Tunable UV/Visible Absorbance Detector LC Module 1 | Long-life deuterium lamp, 2,000 hours | 700000356 WAT052666 | 8005-0702 |

The cross references to the Waters part numbers listed here serve as a recommendation that the Agilent products are viable alternatives to Waters products. Agilent products are compatible with the corresponding Waters instruments, although in some cases, the Agilent products may have slightly different designs as compared to the Waters counterparts. All Agilent supplies are backed by the Agilent 90-day money-back warranty.



Long-life deuterium lamp, 8005-0705



Long-life deuterium lamp, 8005-0704

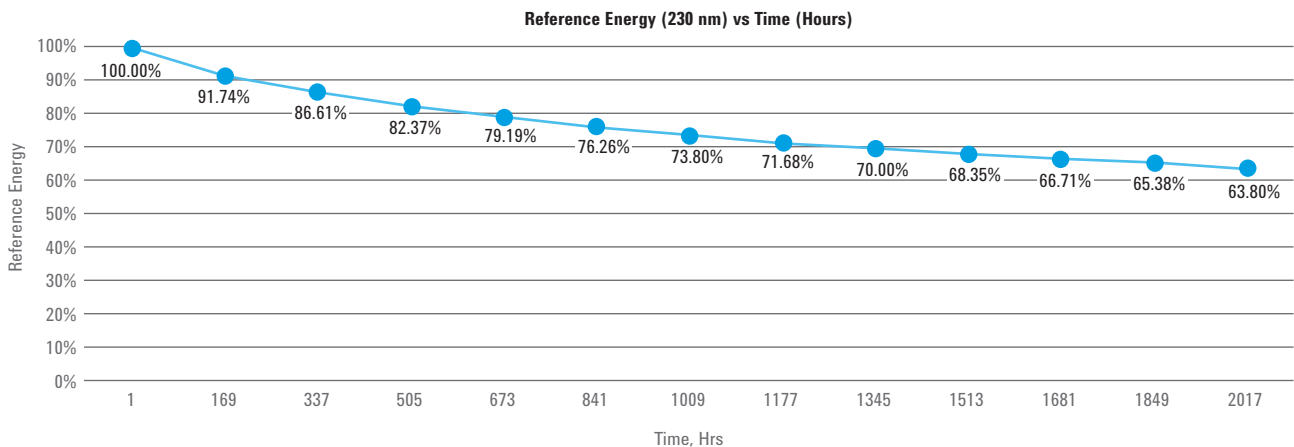


Long-life deuterium lamp, 8005-0702

Updated UV Lamp for Alliance Detectors

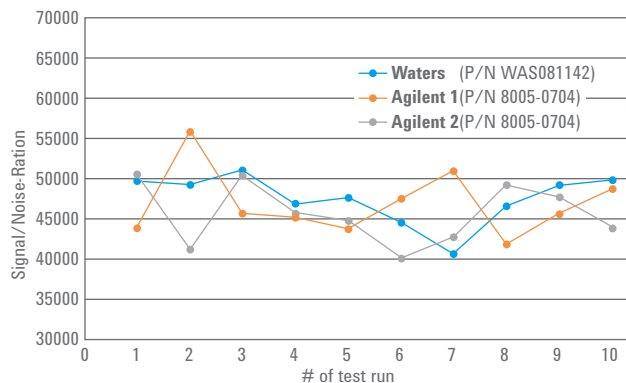
- Precise alignment to fit optical detector configuration to maximize intensity and signal to noise ratio
- Guaranteed long lifetime to lower the cost of ownership
- Certified and traceable production process
- Automated manufacturing process for minimal variations between lamps
- Uniform thickness and highest-quality material of lamp glass for consistent light spectra
- Rigorous testing on original instruments
- Double-insulated cable to meet stringent safety regulations
- Sturdy cap design for better usability

Lifetime: reference energy for the 8005-0704 well above 50% of the initial energy after 2,000 hours



The Agilent-specific cathode coating process leads to less intensity drop over time, and extends lamp lifetime by more than 50%. All Agilent long-life deuterium lamps are guaranteed to have a lifetime greater than 2,000 hours.

Average signal to noise values over ten injections on new lamps are comparable to the Waters lamps





As easy as closing a lever: The proprietary Agilent design features a spring-loaded mechanism for zero dead volume and a sure, tight connection.



TIPS & TOOLS

In a tight spot?

Try an A-Line Quick Turn fitting

For instrument connections that are too tight for Quick Connect fittings, you can rely on Agilent A-Line Quick Turn fittings. Like our Quick Connect fittings, they leverage a proprietary spring-loaded design for zero dead volume and a sure connection.

Note: These fittings can only be used with capillaries using a long socket. See the ordering information for a list of available capillaries.



Agilent A-Line Quick Connect Fittings

The fast, easy way to more predictable results

Poorly connected flow path fittings are one of the top reasons for peak tailing, peak broadening, split peaks, and carryover. That's because dead volume or micro-leakage can cause extra-column volume, which hinders the performance and reproducibility of HPLC and UHPLC.

Conventional fittings are non-adjustable, require specialized wrenches to install, and are incompatible with different column brands. They are also subject to over-tightening, which can cause the fitting to get stuck in the column.

Other manufacturers offer finger-tight fittings; however, these fittings usually have polymeric ferrules that lack grip strength at high pressures. As a result, you must constantly check for leaks and re-tighten the fittings to avoid tubing slippage and dead volume.

Save time, reduce cost, and minimize troubleshooting with A-Line Quick Connect fittings

With a Quick Connect fitting, you can be sure you're getting a perfect column connection, every time.

- **Truly finger-tight connection:** No special training is needed, so differences in user style will not affect your chromatography
- **Spring-loaded design** pushes the tubing against the receiving port for zero-dead-volume connections
- **Compatibility with all types of columns** by simply adjusting the ferrule
- **Reusable for more than 200 reconnections:** Quick Connect fittings are long lasting, reseal tightly, and are stable up to 1,300 bar (18,850 psi)
- **Fast, easy connections** that save you time and trouble—particularly during method development and column testing

"Now everyone in the lab gets an easy, accurate column connection."

PHARMACEUTICAL COMPANY

A-Line Quick Turn Parts

| Description | Part No. |
|--|-----------------|
| Fittings and Ferrules | |
| Quick Turn LC fitting | 5067-5966 |
| Front ferrule | 5043-0924 |
| Capillaries | |
| Stainless steel, 0.075 x 105 mm long socket | 5500-1198 |
| Stainless steel, 0.075 x 150 mm long socket | 5500-1232 |
| Stainless steel, 0.12 x 105 mm long socket | 5500-1188 |
| Stainless steel, 0.12 x 150 mm long socket | 5500-1189 |
| Stainless steel, 0.12 x 180 mm long socket | 5500-1233 |
| Stainless steel, 0.12 x 200 mm long socket | 5500-1190 |
| Stainless steel, 0.12 x 280 mm long socket | 5500-1191 |
| Stainless steel, 0.12 x 500 mm long socket | 5500-1192 |
| Stainless steel, 0.17 x 105 mm long socket | 5500-1193 |
| Stainless steel, 0.17 x 150 mm long socket | 5500-1194 |
| Stainless steel, 0.17 x 180 mm long socket | 5500-1234 |
| Stainless steel, 0.17 x 200 mm long socket | 5500-1195 |
| Stainless steel, 0.17 x 280 mm long socket | 5500-1196 |
| Stainless steel, 0.17 x 380 mm long socket | 5500-1235 |
| Stainless steel, 0.17 x 400 mm long socket | 5500-1236 |
| Stainless steel, 0.17 x 500 mm long socket | 5500-1197 |
| Stainless steel, 0.17 x 700 mm long socket | 5500-1237 |
| NEW Stainless steel, 0.25 mm x 105 mm long socket with a female connection | 5500-1261 |
| NEW Stainless steel, 0.25 mm x 150 mm long socket | 5500-1262 |
| NEW Stainless steel, 0.25 mm x 400 mm long socket | 5500-1263 |

Note: A-Line Quick Turn fittings require the capillaries specified in this table. 0.25 mm id solutions are optimized for Waters Alliance.

A-Line Quick Connect Fittings

| Description | Part No. |
|---|-----------------|
| Assemblies | |
| Stainless steel, 0.075 x 105 mm | 5067-5961 |
| Stainless steel, 0.075 x 150 mm | 5067-6163 |
| Stainless steel, 0.075 x 220 mm | 5067-6164 |
| Stainless steel, 0.075 x 280 mm | 5067-6165 |
| Stainless steel, 0.12 x 105 mm | 5067-5957 |
| Stainless steel, 0.12 x 150 mm | 5067-5958 |
| Stainless steel, 0.12 x 220 mm | 5067-5959 |
| Stainless steel, 0.12 x 280 mm | 5067-5960 |
| Stainless steel, 0.17 x 105 mm | 5067-6166 |
| Stainless steel, 0.17 x 150 mm | 5067-6167 |
| Stainless steel, 0.17 x 220 mm | 5067-6168 |
| Stainless steel, 0.17 x 280 mm | 5067-6169 |
| NEW Stainless steel, 0.25 mm x 105 mm, with a female connection | 5067-6210 |
| Note: Each assembly is equipped with a Quick Connect fitting, a capillary and a Swagelok fitting or with a female connection where specified. 0.25 mm id solutions are optimized for Waters Alliance. | |
| Fittings and Ferrules | |
| Quick Connect LC fitting | 5067-5965 |
| Front ferrule | 5043-0924 |
| Capillaries | |
| Stainless steel, 0.075 x 105 mm | 5500-1174 |
| Stainless steel, 0.075 x 150 mm | 5500-1175 |
| Stainless steel, 0.075 x 220 mm | 5500-1176 |
| Stainless steel, 0.075 x 250 mm | 5500-1177 |
| Stainless steel, 0.075 x 280 mm | 5500-1178 |
| Stainless steel, 0.12 x 105 mm | 5500-1173 |
| Stainless steel, 0.12 x 150 mm | 5500-1172 |
| Stainless steel, 0.12 x 220 mm | 5500-1171 |
| Stainless steel, 0.12 x 280 mm | 5500-1170 |
| Stainless steel, 0.12 x 400 mm | 5500-1179 |
| Stainless steel, 0.12 x 500 mm | 5500-1180 |
| Stainless steel, 0.17 x 105 mm | 5500-1181 |
| Stainless steel, 0.17 x 150 mm | 5500-1182 |
| Stainless steel, 0.17 x 220 mm | 5500-1183 |
| Stainless steel, 0.17 x 280 mm | 5500-1230 |
| Stainless steel, 0.17 x 500 mm | 5500-1231 |
| NEW Stainless steel, 0.25 mm x 105 mm, with a female connection | 5500-1258 |
| NEW Stainless steel, 0.25 mm x 150 mm | 5500-1259 |
| NEW Stainless steel, 0.25 mm x 400 mm | 5500-1260 |

NOTE: The A-Line Quick Connect fitting can only be equipped with an A-Line capillary specified in this table. The A-Line capillary is designed with a spring and a holder. 0.25 mm id solutions are optimized for Waters Alliance.

Agilent A-Line Stay Safe Caps

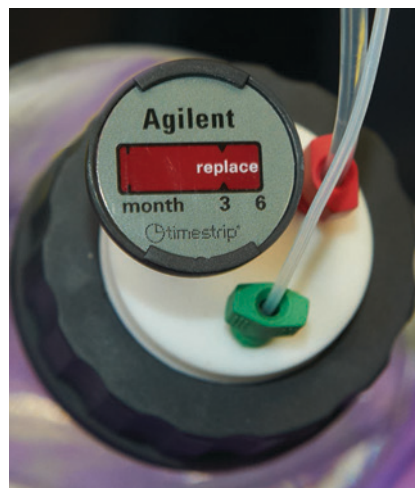
- Stop 99.9% of solvents leaching into the air to protect your health
- Prevent solvent decomposition and protect your chromatographic results
- Ensure the long-term consistency of your mobile phase and chromatographic results

Because no one wants to worry about solvents leaching into the air

Acetonitrile and methanol are just two of the toxic compounds you may be exposed to every day. Fortunately, you can count on Agilent A-Line Stay Safe caps to stop 99.9% of solvents from leaching into the air—protecting you and your coworkers. But that's not all...

A-Line Stay Safe caps also promote mobile phase consistency

Over time, solvent composition can change, affecting your chromatographic results. Storing solvents with airtight Stay Safe caps prevents this problem, ensuring the long-term consistency of your mobile phase and chromatographic results.







Stay Safe caps include a time strip that tells you when the venting valve needs to be replaced. We recommend changing the A-Line venting valve or the A-Line charcoal filter after six months of usage under the following conditions:

- 1 mL/min
- 8 hours per day
- 5 days per week
- Under typical lab conditions at 20 °C



Through innovative design Agilent A-Line Stay Safe caps stop 99.9% of solvents from leaching into the air, making your lab a safer workplace.

A-Line Stay Safe Caps

| Description | Ports | | | | Part No. |
|---|--|------|--------|-------|-----------|
| | Fittings | Vent | Filter | Waste | |
|  GL45 with 1 port 1 vent value with time strip (5043-1190) | 1 x 3.2 mm | 1 | | | 5043-1217 |
|  GL45 with 2 ports 1 vent value with time strip (5043-1190) | 2 x 3.2 mm | 1 | | | 5043-1218 |
|  GL45 with 3 ports 1 vent value with time strip (5043-1190) | 3 x 3.2 mm | 1 | | | 5043-1219 |
|  GL45 with 4 ports 1 leak hose | 4 (2 x 3.2 mm, 1 x 2.3 mm, 1 x 1.6 mm) | | 1 | 1 | 5043-1220 |

Kits

| Description | Part No. |
|---|-----------|
| 6 L waste can (5043-1196) Stay Safe cap GL45 with 4 ports (5043-1220) | 5043-1221 |
| Infinity Stay Safe cap kit—contains: 4 caps • 3 (5043-1217) • 1 (5043-1218) 4 venting valves with time strip (5043-1190) 4 fittings (3.2 mm) | 5043-1222 |

For Fitting Ports

| Description | Part No. |
|--------------------------------------|-----------|
| Fitting for 3.2 mm tubing, PFA, 2/pk | 5043-1216 |
| Fitting for 2.3 mm tubing, PFA, 2/pk | 5043-1215 |
| Fitting for 1.6 mm tubing, PFA, 2/pk | 5043-1214 |
| Screw plug 1/8 in, PTFE, 2/pk | 5043-1198 |

For Vent Ports

| Description | Part No. |
|---|-----------|
| Venting valve with time strip, PTFE, 1 µm | 5043-1190 |

For Filter Port

| Description | Part No. |
|--|-----------|
| Charcoal filter with time strip (58 g) for waste container | 5043-1193 |

For Waste Port

| Description | Part No. |
|-------------------------------|-----------|
| 2 ports waste collector, PTFE | 5043-1207 |
| Screw plug 1/4 in, PTFE | 5043-1195 |

Miscellaneous

| Description | Part No. |
|--|-----------|
| Thread adapter, PTFE, GL45(M) - GL40(F) | 5043-1191 |
| Thread adapter, PTFE, GL45(M) - GPI38-430(F) | 5043-1192 |
| 6 L waste can, GL45 | 5043-1196 |

PROPER INSTRUMENT MAINTENANCE FOR ALLIANCE SYSTEMS

Autosampler Routine Maintenance

Routine autosampler maintenance should be performed on a regular basis to keep your HPLC system performing at its optimum level. You can perform all maintenance procedures at once or as needed. Some parts may need to be replaced more often than others, depending upon your application and solvent preparation procedures.

Regular autosampler maintenance helps lower operating costs and generates precise results with the utmost confidence. By following a regular maintenance routine, you can count on maximum uptime during the life of your autosampler.

Waters Autosampler Routine Maintenance Procedures

- Replace the metering syringe
- Rebuild the injector seal pack and replace the needle
- Rebuild the high-pressure motorized valves
- Replace the inline filter insert
- Adjust the seal pack seal valves

Waters Autosampler Routine Maintenance and Troubleshooting

| Symptom | Cause | Solution |
|---|--|--|
| Poor injection reproducibility | Low sample level in vials | Confirm sample level in the vials is at minimum a quarter full |
| | Worn metering syringe | Replace the metering syringe assembly |
| | Worn high-pressure motorized valve seals | Rebuild the high-pressure motorized valves |
| | Leaking waste valve | Replace the waste valve |
| | Worn injector seals | Rebuild the injector seal pack, replace the needle, and adjust the seal pack seal valves |
| Leaking syringe | Worn metering syringe | Replace the metering syringe assembly |
| Sample carryover | Needlewash solvent exhausted | Refill the needlewash solvent reservoir |
| | Dirty needlewash seals | Rebuild the seal pack and adjust seal pack seal valves |
| | Faulty needlewash valve | Replace the needlewash valve |
| Sample vials filling during injection cycle | Worn high-pressure motorized valve seal | Rebuild the high-pressure motorized valves |
| Low peak response | Low sample level in vials | Confirm sample level in the vials is at minimum a quarter full |
| | Worn metering syringe | Replace the metering syringe assembly |
| | Worn high-pressure motorized valve seals | Rebuild the high-pressure motorized valves |
| | Leaking waste valve | Replace the waste valve |
| | Worn injector seals | Rebuild the injector seal pack, replace the needle, and adjust the seal pack seal valves |



Sapphire plunger assembly, 8005-0523

HPLC Pump Routine Maintenance

Routine pump maintenance should be performed on a regular basis to keep your HPLC system performing at its optimum level. You can perform all maintenance procedures at once or as needed. Some parts may need to be replaced more often than others depending upon your application and solvent preparation procedures.

Regular pump maintenance helps lower operating costs and generates precise results with the utmost confidence. By following a regular maintenance routine, you can count on maximum uptime and a steady, accurate solvent flow for the life of the pump.

TIPS & TOOLS

Pump seals should be replaced when there are leaks on the bottom of the pump head, when retention times are inconsistent, or when the pressure ripple is unstable.

Waters Pump Routine Maintenance Procedures

- Replace the seals and plungers (or pistons)
- Replace the inline filter insert
- Replace the check valve cartridges
- Replace the seal wash seals and tube seals
- Replace the solvent inlet frits

Waters Pump Routine Maintenance Procedures

| Symptom | Cause | Solution |
|--|--------------------------------|--|
| Pressure ripple unstable | Dirty check valve cartridge(s) | Run static leak test to verify and exchange the check valve cartridge(s) |
| | Leak on pump head | Run static leak test to verify and exchange the inline filter insert and plunger (or piston) seals |
| Gradient performance problems Intermittent pressure fluctuations | Blocked solvent filter(s) | Change the solvent filter(s) |
| A pressure drop of greater than 10 bar (150 psi) across the inline filter (5 mL/min H ₂ O during wet priming) | Dirty inline filter | Exchange the inline filter insert |
| Leaks at lower pump head side Unstable retention time Pressure ripple unstable | High seal wear | Run leak test to verify and exchange the pump seals and inline filter insert |
| Seal lifetime shorter than normally expected | Scratch on plunger | Check plungers while changing the seals Exchange the plungers if damaged or scratched |
| Loss of wash solvent | Leaky wash seals | Exchange the wash seals |

Troubleshooting Guide for HPLC Systems

The at-a-glance tables can help you pinpoint and solve the most common problems for your HPLC systems and ensure maximum instrument uptime and productivity.

HPLC Troubleshooting

| Symptom Type | Possible Cause | Solution |
|-----------------------------------|---|--|
| Baseline disturbance at void time | Positive/negative—Difference in refractive index of injection solvent | Use mobile phase for sample solvent |
| Detector leaks | Plugged inlet frit | Replace seals/gaskets |
| Drifting baseline | Positive direction—Contaminant buildup/elution | Flush column, clean up sample, use pure solvents |
| | Positive/negative—Difference in refractive index of injection solvent | Use mobile phase for sample solvent |
| | Negative direction (gradient)—Absorbance of "A" mobile phase solvent | Use non-absorbing or HPLC-grade or better solvent |
| | Positive direction (gradient)—Absorbance of "B" mobile phase solvent | Use non-absorbing or HPLC-grade or better solvent |
| | Random—Temperature changes | Insulate column and tubing |
| | Random—Temperature changes | Thermostat column and tubing |
| | Wavy or undulating—Temperature changes in room | Monitor room temperature and control |
| Ghost peaks | Peaks from previous injection | Flush column to remove contaminants |
| | Contamination | Sample cleanup or pre-fractionation |
| | Unknown interferences in samples | Sample cleanup or pre-fractionation |
| | Ion-pair—Upset equilibrium | Prepare sample in actual mobile phase to minimize disturbance |
| | Peptide mapping—Oxidation of TFA | Prepare fresh daily; use anti-oxidant |
| | Reversed-phase—Contaminated water | Check suitability of water by running different amount through reversed-phase column and measure peak height with elution; use HPLC grade solvents |
| | Spikes—Bubbles in solvent | De-gas solvents |
| High column backpressure | Column blockage, adsorbed sample | Better sample cleanup; use guard column |
| | Mobile phase viscosity too high | Use lower viscosity solvents or higher temperature |
| | Particle size too small | Use larger d_p packing |
| | Plugged inlet frit | Replace column |
| | Plugged inlet frit | Reverse solvent flow |

(Continued)

HPLC Troubleshooting

| Symptom Type | Possible Cause | Solution |
|---------------------------------------|---|--|
| Leaks | Subtle—White powder at fitting/loose fitting | Tighten fittings, cut tubing, or replace ferrules |
| Leaks, injection valve | Catastrophic—Worn valve rotor | Replace rotor in valve |
| Leaks, column or other fittings | Catastrophic—Loose fittings | Tighten or replace fittings |
| Leak, pump | Catastrophic—Pump seal failure | Replace pump seal |
| Negative peaks | RI detector—Solute refractive index less than solvent | No problem; reverse polarity to make positive |
| | UV detector—Solute absorbance less than mobile phase | Use mobile phase with lower UV absorbance; do not recycle solvent too many times |
| Noisy baseline | Random—Contaminant buildup | Flush column; clean up sample; use HPLC-grade solvent |
| | Continuous—Detector lamp problem | Replace detector lamp |
| | Occasional—External electrical interference | Use voltage stabilizer for LC system |
| Peak doubling | Sample volume too large | Reduce the volume e.g. by half and re-inject |
| | Injection solvent too strong | Use weaker injection solvent or mobile phase |
| | Blocked frit | Replace and use 0.5 μm porosity inline filter |
| | Column void or channeling | Replace column; for some columns, fill in void with packing |
| | Unswep injector flowpath | Replace injector rotor |
| | Void at head of column | Replace column, top off column with packing |
| | Column overloaded with sample | Use higher capacity stationary phase Increase column diameter Decrease sample size |
| | Single peak—Interfering components | Sample cleanup; pre-fractionation |
| | Beginning of peak doubling | See “peak doubling” |
| | Peak tailing | Unswep dead volumes |
| Basic compounds—Silanol interactions | | Choose endcapped bonded phase Switch to polymeric phase |
| Basic substances—Silanol interactions | | Use stronger mobile phase or add competing base (e.g. TMA) |
| Silica-based—Column degradation | | Use specialty column; polymeric column or sterically protected |

(Continued)

HPLC Troubleshooting

| Symptom Type | Possible Cause | Solution |
|--|--|--|
| Peaks are broad | Injection volume too large | Decrease solvent strength of injection solvent to focus solute |
| | Peak dispersion in injector valve | Introduce air bubble in front/back of sample to decrease dispersion |
| | Sampling rate of data system too slow | Increase frequency of sampling |
| | Slow detector time constant | Adjust time constant to match peak width |
| | Mobile phase viscosity too high | Increase column temperature |
| | Detector cell volume too large | Use smallest possible cell volume with no heat exchanger in system |
| | Injector volume too large | Decrease injection volume |
| | Long retention times | Use gradient elution or stronger mobile phase |
| Pressure fluctuation | Leaky check valve | Replace check valve |
| | Pump seal leaks | Replace pump seals |
| | Buildup of particulates | Filter sample; inline filter; filter mobile phase |
| Pressure increasing | Buildup of particulates | Filter sample; inline filter; filter mobile phase |
| | Water/organic systems—Buffer precipitation | Test buffer-organic mixtures; ensure compatibility |
| Retention beyond total permeation volume | Size exclusion—Specific interactions | Add mobile phase modifiers or change solvent |
| Retention times changing | Column temperature varying | Thermostat column; insulate column; ensure lab temperature constant |
| | Equilibration time insufficient with gradient run or changes in isocratic mobile phase | Make sure at least 10 column volumes pass through column after solvent change or gradient conclusion |
| | Selective evaporation of mobile phase component | Less vigorous helium sparging; keep solvent reservoirs covered; prepare fresh mobile phase |
| | Buffer capacity insufficient | Use >20 mM concentration of buffer |
| | Inconsistent online mobile phase mixing | Ensure gradient system delivering constant composition; check vs. manual prep of mobile phase |
| | Contamination buildup | Occasionally flush column with strong solvent to remove contaminants |
| | First few injections—Adsorption on active sites | Condition column by initial injection of concentrated sample |
| Retention times decreasing | Flow rate increasing | Check pump to make sure correct; if not, reset |
| | Column overloaded with sample | Decrease sample size |
| | Loss of bonded stationary phase | Keep mobile phase pH between 2 and 8.5 |

(Continued)

HPLC Troubleshooting

| Symptom Type | Possible Cause | Solution |
|---|--|---|
| Retention times increasing | Flow rate is slowing | Fix leaks in liquid lines, replace pump seals, check for pump cavitation or air bubbles |
| | Active sites on silica packing | Use mobile phase modifier |
| | Loss of bonded stationary phase | Keep mobile phase pH between 2 and 8.5 |
| | Mobile phase composition changing | Make sure mobile phase container is covered |
| | Active sites on silica packing | Add competing base to mobile phase |
| | Active sites on silica packing | Use higher coverage packing for stationary phase |
| Sensitivity problem | Peaks are outside linear range of detector | Dilute/concentrate to bring into linear region |
| | First few sample injections—Absorption of sample in loop or column | Condition loop/column with concentrated sample |
| | Autosampler flow lines blocked | Check flow and make sure there are no blockages |
| | Injector sample loop underfilled | Make sure that loop is overfilled with sample |
| | Sample-related losses during preparation | Use internal standard during sample prep; optimize sample prep method |
| Slow column equilibration times (ion-pairing) | Equilibration time slow for long-chain ion-pairing reagents | Use shorter alkyl chain ion-pair reagent |



515 pump PM kit, 8005-0913



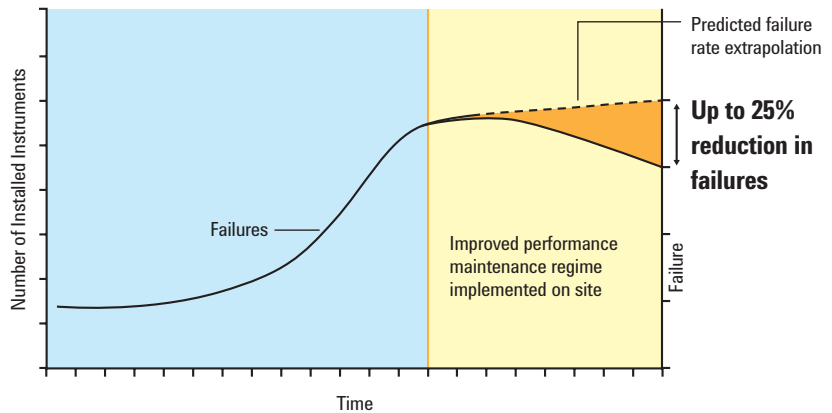
2960/2965 PM kit, 8005-0915

Maintenance reduces downtime up to 25%

Performance maintenance kits make it easier to keep your instruments running at peak performance

Many instrument failures are not caused by functional breakdowns, but by a lack of performance maintenance. As suggested by a recent study, a regular performance maintenance program can reduce instrument failure rates up to 25%, and is critical to ensure optimal overall system operations.

The Effect of Performance Maintenance



Agilent PM kits contain the normal wear parts, tools for disassembly/reassembly, and instructions necessary to keep your systems operating at peak performance. The kits provide a convenient, cost-effective way to make sure your instruments are properly maintained. Each kit contains all the parts you need to:

- Boost system efficiency and reliability
- Reduce unplanned downtime and repair costs
- Increase accuracy by enhancing precision and sensitivity
- Comply with regulatory requirements, or meet quality accreditation standards
- Extend instrument life
- Eliminate individual part ordering

PM kits are available for pumps, valves, autosamplers, and HPLC systems.

2695 Separations Module, Enhanced PM

| Description | Unit | Similar to Waters Part No. | Part No. |
|---|------|----------------------------|------------------|
| 2695 Separations Module, Enhanced PM | | | 8005-0999 |
| Piston Seal (standard) | 2/pk | WAT270938 | 8005-0535 |
| Piston | 1/pk | WAT270959 | 8005-0538 |
| Inline Filter Element | 1/pk | WAT088084 | 8005-0424 |
| Wash Tube Seal Replacement Kit | 4/pk | WAT270940 | 8005-0557 |
| Seal Wash Plunger Seal | 2/pk | WAT271018 | 8005-0540 |
| Head Face Seals | 4/pk | WAT270939 | 8005-0536 |
| Check Valve Replacement Kit | 2/pk | WAT270941 | 8005-0537 |
| Solvent Filter Assembly | 1/pk | WAT025531 | 8005-0849 |
| M715 Seal, HPMV | 1/pk | WAT045454 | 8005-0425 |
| Seal, Insert | 1/pk | WAT021132 | 8005-0426 |
| O-ring, PTFE, 440/441 | 2/pk | WAT097387 | 8005-0427 |
| Seal Pack Rebuild Kit | 1/pk | WAT271019 | 8005-0916 |
| Syringe Assembly, 250 μ L | 1/pk | WAT073109 | 8005-0419 |
| Battery, 3 V | 1/pk | WAT80443 | 8005-0428 |

1525 Pump, 225 μ L Head

| Module/System | Similar to Waters Part No. | Part No. |
|--|----------------------------|------------------|
| 1525 Pump, 225 μL head | | 8005-0429 |
| Extended Flow Plunger Seal, 225 μ L | 700002282 | N/A |
| Outlet Check Valve and Housing, 225 μ L | WAT025216 | 8005-0430 |
| Reference Valve Rebuild Kit | WAT025746 | 8005-0911 |
| Extended Flow Plunger | WAT60304 | 8005-0431 |
| Inlet Check Valve and Housing | WAT60307 | 8005-0432 |
| Solvent Filter | WAT025531 | 8005-0849 |

1525 Pump, 100 μ L Head

| Module/System | Similar to Waters Part No. | Part No. |
|--|-----------------------------------|------------------|
| 1525 Pump, 100 μL head | | 8005-0433 |
| 100 Plunger Seal, Clear | WAT022934 | 8005-0524 |
| Check Valve Cartridges, 2/pk | WAT700000254 | 8005-0513 |
| Plunger | WAT207069 | 8005-0523 |
| Reference Valve Rebuild Kit | WAT025746 | 8005-0911 |
| Solvent Filter | WAT025531 | 8005-0849 |

1525 Pump, 50 μ L Head

| Module/System | Similar to Waters Part No. | Part No. |
|---|-----------------------------------|------------------|
| 1525 Pump, 50 μL head | | 8005-0434 |
| 1525 Micro Seal Kit | WAT205000202 | |
| Plunger | WAT30547 | 8005-0549 |
| Check Valve Cartridges, 2/pk | WAT700000254 | 8005-0513 |
| Solvent Filter | WAT025531 | 8005-0849 |

1515 Pump, 100 μ L Head

| Module/System | Similar to Waters Part No. | Part No. |
|--|-----------------------------------|------------------|
| 1515 Pump, 100 μL head | | 8005-0435 |
| 100 Plunger Seal, Clear | WAT022934 | 8005-0524 |
| Plunger | WAT207069 | 8005-0523 |
| Check Valve Cartridges, 2/pk | WAT700000254 | 8005-0513 |
| Reference Valve Rebuild Kit | WAT025746 | 8005-0911 |
| Solvent Filter | WAT025531 | 8005-0849 |

2695 Separations Module, Standard PM

| Module/System | Similar to Waters Part No. | Part No. |
|---|-----------------------------------|------------------|
| 2695 Separations Module, Standard PM | | 8005-0998 |
| Piston Seal, Standard, 2/pk | WAT270938 | 8005-0535 |
| Inline Filter Element | WAT088084 | 8005-0424 |
| Seal Wash Plunger Seal, 2/pk | WAT271018 | 8005-0540 |
| Seal, Insert | WAT021132 | 8004-0426 |
| Seal Pack Rebuild Kit With Needle | WAT271019 | 8005-0916 |
| Syringe Seal Kit (syringe seals and plunger tips) | WAT073210 | 8005-0437 |
| Battery, 3 V | WAT80443 | 8005-0428 |

1525 Pump, 100 µL Head, Micro

| Module/System | Similar to Waters Part No. | Part No. |
|--------------------------------------|-----------------------------------|------------------|
| 1525 Pump, 100 µL head, micro | | 8005-0440 |
| Extended Flow Plunger Seal | 700002282 | |
| Outlet Check Valve and Housing | WAT25216 | 8005-0430 |
| 100 Plunger Seal, Clear | WAT022934 | 8005-0524 |

1525 Pump, 50 µL Head, Micro

| Module/System | Similar to Waters Part No. | Part No. |
|-------------------------------------|-----------------------------------|------------------|
| 1525 Pump, 50 µL Head, Micro | | 8005-0439 |
| 1525 Micro Seal Kit | WAT205000202 | 8005-0524 |

Performance Maintenance Kits

| Model | Description | Kit Contents | Similar to Waters Part No. | Part No. |
|---|--|---|----------------------------|-----------|
| Performance Maintenance Kits for Rheodyne Valves | | | | |
| Rheodyne Injector, 3725(i) | Performance Maintenance Kit for Rheodyne 3725(i) Injector Valves | Rotor seal (PEEK), stator face assembly (PEEK), isolation seal, needle guide, needle port cleaner, hex key (5/64 in), hex key (9/64 in), instructions | 201000116 | 8005-0901 |
| Rheodyne Injector, 7010 | Performance Maintenance Kit for Rheodyne 7010 Injector Valves | Isolation seal, Rotor seal (Vespel), hex key (5/64 in), hex key (9/64 in), instructions | 201000117 | 8005-0902 |
| Rheodyne Injector, 7125 and 7126 | Performance Maintenance Kit for Rheodyne 7125 and 7126 Injector Valves | Rotor seal (Vespel), stator face assembly (PEEK/ceramic), isolation seal, needle guide, needle port cleaner, hex key (5/64 in), hex key (9/64 in), instructions | 201000118 | 8005-0903 |
| Rheodyne Injector, 7725(i) | Performance Maintenance Kit for Rheodyne 7725(i) Injector Valves | Stator face assembly (PEEK/ceramic), rotor seal (Vespel), isolation seal, needle guide, needle port cleaner, hex key (5/64 in), hex key (9/64 in), instructions | 201000119 | 8005-0904 |
| Rheodyne Injector, 7750E | Performance Maintenance Kit for Rheodyne 7750 Injector Valves | Stator face assembly (PEEK/ceramic), rotor seal (Vespel), isolation seal, hex key (9/64 in), instructions | 201000122 | 8005-0907 |
| Rheodyne Injector, 7750E-075 | Performance Maintenance Kit for Rheodyne 7750E-075 Valves | Rotor seal (PEEK), stator face assembly (PEEK), isolation seal, hex key (9/64 in), instructions | 201000125 | 8005-0908 |
| Rheodyne Injector, 8125 and 8126 | Performance Maintenance Kit for Rheodyne 8125 and 8126 Injector Valves | Stator face assembly (PEEK/ceramic), rotor seal (PEEK), isolation seal, needle guide, needle port cleaner, hex key (5/64 in), hex key (9/64 in), instructions | 201000120 | 8005-0905 |
| Rheodyne Injector, 9125 and 9126 | Performance Maintenance Kit for Rheodyne 9125 and 9126 Injector Valves | Stator face assembly (PEEK/ceramic), rotor seal (Tefzel), isolation seal, needle guide, needle port cleaner, hex key (5/64 in), hex key (9/64 in), instructions | 201000121 | 8005-0906 |

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Rheodyne 7725(i) PM kit, 8005-0904



Rheodyne 9125 PM kit, 8005-0906

Pump Supplies

| Model | Description | Unit | Similar to Waters Part No. | Part No. |
|---|--|------|----------------------------|-----------|
| Plungers and Seals | | | | |
| 2690 Separations Module 2690D Dissolution Separations Module 2695 Separations Module 2695D Dissolution Separations Module 2790 Separations Module 2795 Separations Module 2796 Bioseparations Module | Sapphire plunger assembly, standard | 1/pk | WAT270959 | 8005-0538 |
| 2690 Separations Module 2690D Dissolution Separations Module 2695 Separations Module 2695D Dissolution Separations Module | Plunger seals, clear | 2/pk | 700001326 | 8005-0514 |
| 2690 Separations Module 2690D Dissolution Separations Module 2695 Separations Module 2695D Dissolution Separations Module 2790 Separations Module 2795 Separations Module Alliance GPC/V 2000 Systems | Plunger seal replacement kit, standard, yellow | 2/pk | WAT270938 | 8005-0535 |
| 2690 Separations Module 2690D Dissolution Separations Module 2695 Separations Module 2695D Dissolution Separations Module 2790 Separations Module 2795 Separations Module | Plunger seal replacement kit, black | 2/pk | WAT271066 | 8005-0541 |

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Sapphire plunger assembly, 8005-0538



Plunger seal replacement kit, 8005-0535



Plunger seal replacement kit, 8005-0541

Pump Supplies

| Model | Description | Unit | Similar to Waters Part No. | Part No. |
|---|--|------|----------------------------|-----------|
| Plungers and Seals | | | | |
| 2690 Separations Module 2690D Dissolution Separations Module 2695 Separations Module 2695D Dissolution Separations Module 2790 Separations Module 2795 Separations Module Alliance GPC/V 2000 Systems | Face seals replacement kit | 4/pk | WAT270939 | 8005-0536 |
| 2690 Separations Module 2690D Dissolution Separations Module 2695 Separations Module 2695D Dissolution Separations Module 2790 Separations Module 2795 Separations Module Alliance GPC/V 2000 Systems | Seal wash face seal kit | 1/pk | WAT271017 | 8005-0539 |
| 2796 Bioseparations Module | Seal wash, plunger seal | 4/pk | 700002258 | 8005-0516 |
| 2796 Bioseparations Module | Plunger seal, standard | 2/pk | 700002257 | 8005-0515 |
| 2690 Separations Module 2690D Dissolution Separations Module 2695 Separations Module 2695D Dissolution Separations Module 2790 Separations Module 2795 Separations Module Alliance GPC/V 2000 Systems | Seal wash plunger seal replacement kit | 2/pk | WAT271018 | 8005-0540 |
| 510 HPLC Pump | Sapphire plunger | 1/pk | WAT025656 | 8005-0527 |
| 600 MultiSolvent Delivery System 610 PowerLine Isocratic Pump LC Module 1 | Oriented sapphire plunger | 1/pk | WAT069511 | 8005-0533 |

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Face seals replacement kit, 8005-0536



Seal wash face seal kit, 8005-0539

Pump Supplies

| Model | Description | Unit | Similar to Waters Part No. | Part No. |
|---|--|-------------|-----------------------------------|-----------------|
| Plungers and Seals | | | | |
| 510 HPLC Pump 515 HPLC Pump 600 MultiSolvent Delivery System LC Module 1 | Plunger seal, graphite-filled, PTFE (GFP), black | 1/pk | WAT026613 | 8005-0529 |
| 510 HPLC Pump 515 HPLC Pump 600 MultiSolvent Delivery System 610 PowerLine Isocratic Pump LC Module 1 | Plunger seal, black, 225 µL | 1/pk | WAT026644 | 8005-0530 |
| 616 LC System 626 LC System | Sapphire plunger | 1/pk | WAT031788 | 8005-0531 |
| 1515 HPLC Pump 1525 HPLC Pump 515 HPLC Pump | Sapphire plunger assembly | 1/pk | WAS207069 | 8005-0523 |
| 1515 HPLC Pump 1525 HPLC Pump | Plunger seal, clear | 1/pk | WAT022934 | 8005-0524 |
| 510 HPLC Pump 515 HPLC Pump 600 MultiSolvent Delivery System 610 PowerLine Isocratic Pump LC Module 1 | Plunger seal, clear | 4/pk | WAT022946 | 8005-0525 |
| Check Valves and Cartridges | | | | |
| 2690 Separations Module 2690D Dissolution Separations Module 2695 Separations Module 2695D Dissolution Separations Module 2790 Separations Module 2795 Separations Module Alliance GPC/V 2000 Systems | Check valve cartridge replacement kit | 2/pk | WAT270941 | 8005-0537 |

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Pump Supplies

| Model | Description | Unit | Similar to Waters Part No. | Part No. |
|--------------------------------------|--|------|----------------------------|-----------|
| Check Valves and Cartridges | | | | |
| 626 LC System | Check valve cartridge | 1/pk | WAT024120 | 8005-0526 |
| 1515 HPLC Pump | | | | |
| 1525 HPLC Pump | | | | |
| 2695 Separations Module | | | | |
| 2695D Dissolution Separations Module | | | | |
| 2795 Separations Module | Check valve cartridge | 2/pk | 700000254 | 8005-0513 |
| 510 HPLC Pump | | | | |
| 515 HPLC Pump | | | | |
| 600 MultiSolvent Delivery System | | | | |
| 610 PowerLine Isocratic Pump | | | | |
| LC Module 1 | | | | |
| 1525 HPLC Pump | | | | |
| 2695 Separations Module | | | | |
| 2695D Dissolution Separations Module | Check valve cartridge | 1/pk | 700002399 | 8005-0508 |
| 2795 Separations Module | | | | |
| 515 HPLC Pump | | | | |
| 600 MultiSolvent Delivery System | | | | |
| 1515 HPLC Pump | | | | |
| 1525 HPLC Pump | | | | |
| 515 HPLC Pump | Cartridge check valve system | 2/pk | 700000253 | 8005-0512 |
| 600 MultiSolvent Delivery System | | | | |
| LC Module 1 | | | | |
| 510 HPLC Pump | | | | |
| 515 HPLC Pump | | | | |
| 600 MultiSolvent Delivery System | Outlet check valve rebuild kit, 225 µL | 2/pk | WAT026014 | 8005-0528 |
| 610 PowerLine Isocratic Pump | | | | |
| LC Module 1 | | | | |

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Detector Supplies

| Model | Description | Unit | Similar to Waters Part No. | Part No. |
|--------------------------------|-------------|------|----------------------------|-----------|
| 2996 Photodiode Array Detector | Cell gasket | 2/pk | WAT057924 | 8005-0532 |
| 996 Photodiode Array Detector | | | | |

Valve Replacement Parts, 1/pk

| Model | Description | Similar to Waters Part No. | Part No. |
|---|---------------------------------|----------------------------|-----------|
| 2767/2747 Sample Manager Column/Fluidics Organizer | Stator, 6 port, stainless steel | 700001560 | 8005-0601 |
| Rheodyne 7725(i) Injector | Vespel rotor seal | WAT055946 | 8005-0604 |
| 626 LC System Rheodyne 9125 Injector | Tefzel rotor seal | WAT015781 | 8005-0603 |
| 2700 Sample Manager | Rotor seal | WAT272615 | 8005-0605 |
| 2707 Autosampler | Rotor seal | 700003851 | 8005-0602 |

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Rheodyne stator, 8005-0601



Vespel rotor seal, for Rheodyne 7725(i), 8005-0604



Tefzel rotor seal, 8005-0603

Sample Loops, 1/pk

| Model | Volume (µL) | Material | Similar to Waters Part No. | Part No. |
|--------------------------------------|--------------------|-----------------|-----------------------------------|-----------------|
| 2690 Separations Module | 100 | Stainless steel | 430001194 | 8005-0840 |
| 2690D Dissolution Separations Module | | | | |
| 2695 Separations Module | 100 | Stainless steel | 430001194 | 8005-0840 |
| 2695D Dissolution Separations Module | | | | |
| 2695 Separations Module | 200 | Stainless steel | 430001630 | 8005-0841 |
| 2695D Dissolution Separations Module | | | | |
| 2796 Bioseparations Module | 20 | PEEK | 430000782 | 8005-0838 |
| 2796 Bioseparations Module | 100 | PEEK | 430000783 | 8005-0839 |
| 2707 Autosampler | 5 | Stainless steel | 700000683 | 8005-0843 |
| CapLC System | | | | |
| CapLC XE System | 10 | Stainless steel | 700003872 | 8005-0845 |
| 2707 Autosampler | | | | |
| 2707 Autosampler | 20 | Stainless steel | 700000680 | 8005-0842 |
| CapLC System | | | | |
| CapLC XE System | 50 | Stainless steel | 700003928 | 8005-0846 |
| 2707 Autosampler | | | | |
| 2707 Autosampler | 100 | Stainless steel | 700000685 | 8005-0844 |
| CapLC System | | | | |
| CapLC XE System | | | | |

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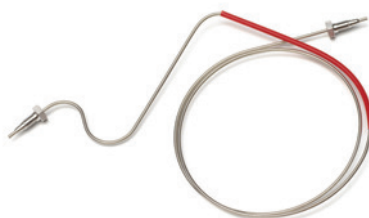
HPLC Capillaries and Capillary Fittings

HPLC Capillaries, 1/pk

| Model | From | To | Material | OD (mm) | ID (mm) | Length (mm) | Fittings | Similar to Waters Part No. | Part No. |
|--|-------------|-------------------------|-----------------|---------|---------|-------------|--|----------------------------|-----------|
| 2690 Separations Module 2690D Dissolution Separations Module 2695 Separations Module 2695D Dissolution Separations Module | Pump | Autosampler | Stainless steel | 1.6 | 0.23 | 760 | With fittings, pre-swaged on both ends | WAT270975 | 8005-0824 |
| 2690 Separations Module 2690D Dissolution Separations Module 2695 Separations Module 2695D Dissolution Separations Module 2790 Separations Module 2795 Separations Module | Autosampler | Column Thermostat Valve | Stainless steel | 1.6 | 0.23 | 760 | With fittings, pre-swaged on both ends | WAT270979 | 8005-0825 |
| 2695 Separations Module 2695D Dissolution Separations Module | Autosampler | Column Thermostat Valve | PEEK | 1.6 | 0.13 | 6,000 | With fittings, non pre-swaged | 430000922 | 8005-0812 |

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Capillary, stainless steel, 8005-0825



Capillary, PEEK, 8005-0812

HPLC Capillaries, 1/pk

| Model | From | To | Material | OD (mm) | ID (mm) | Length (mm) | Fittings | Similar to Waters Part No. | Part No. |
|-----------------|---------------------------------|----------|-----------------|---------|---------|-------------|-------------|----------------------------|------------|
| Alliance | Column | Column | Stainless steel | 1.6 | 0.23 | 3,000 | No fittings | WAT026973 | 8005-0823 |
| | Thermostat Valve | Detector | | | | | | | |
| Alliance | Column | Column | Stainless steel | 1.6 | 0.508 | 3,000 | No fittings | WAT026804 | 8005-0826 |
| | Thermostat Valve | Detector | | | | | | | |
| Alliance | Column | Column | Stainless steel | 1.6 | 1.02 | 3,000 | No fittings | WAT026805 | 8005-0822 |
| | Thermostat Valve | Detector | | | | | | | |
| Fittings | | | | | | | | | |
| Alliance | Compression screws and ferrules | | | | | | | WAT025604 | 8005-0835* |

*This fitting is used with 8005-0823, 8005-0826, and 8005-0822

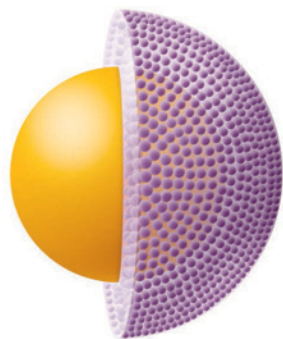


Capillary, stainless steel, 8005-0823

HPLC Fittings, Ferrules, and Unions

| Description | Unit | Similar to Waters Part No. | Part No. |
|--|-------|----------------------------|-----------|
| Compression screw, stainless steel, 1/16 in od | 10/pk | WAT005070 | 8005-0837 |
| Compression screws and ferrules | 5/pk | WAT025604 | 8005-0835 |
| Union, stainless steel, 1/16 in od | 1/pk | WAT097332 | 8005-0836 |

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AGILENT LC COLUMNS AND SUPPLIES FOR SMALL MOLECULE ANALYSIS

Make every HPLC in your lab work harder

The Poroshell 120 column family gives you the benefits of exceptional efficiency—more speed and more resolution—on your conventional HPLC instruments, and great performance on your UHPLC, too.

Poroshell 120 columns provide the speed and resolution of a sub-2 μm column with much less backpressure, and so you can push every analysis further.

To offer you even more choices for refining challenging analyses, the Poroshell 120 family is expanding. Now, there are TWO particle sizes (2.7 and 4 μm) so you can choose the option to best fit your needs. Also, with 12 chemistries available in 2.7 μm and seven chemistries in 4 μm , you have the method development flexibility you are looking for.

- **Multiple chemistries** for flexible method development
- **Larger particle size** with low backpressure—ideal for the pressure limitations of the Alliance system
- **Large frit porosity** with less likelihood of clogging with dirty samples (such as with food, environmental, or samples in plasma or urine)
- **Long lifetime** with pressure limits up to 600 bar—an ideal fit for an Alliance system
- **Scalable particle sizes** in 2.7 μm and 4 μm for drop in replacements into existing methods





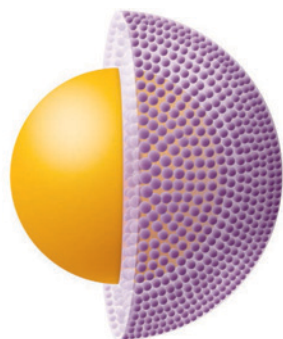
Poroshell 120, 2.7 μm —for UHPLC performance on Waters Alliance HPLC

Agilent Poroshell 120 columns are based on a 2.7 μm particle with a 1.7 μm solid core and 0.5 μm porous outer layer. This small particle size provides high efficiency, similar to sub-2 μm columns, but with 40-50% less pressure. These high efficiency, high resolution columns can be used on any type of LC. The porous outer layer and solid core limit diffusion distance, together with narrow particle size distribution create increased efficiencies enabling very fast, high resolution separations. The same principles are used in columns from the AdvanceBio family, ideal for fast, high-resolution separations of biomolecules.

Poroshell 120, 4 μm —drop-in replacement for high performance without higher pressure

These columns greatly expand the Poroshell 120 family, providing scalable solutions for chromatographers and method developers. The robust 4 μm columns deliver easy drop-in method replacement, with column backpressures typically below 200 bar, and efficiencies nearly double those of traditional, totally porous 5 μm columns. If you want easy performance increase, simply bring Poroshell 120, 4 μm columns into your Waters method.

With its exceptional flexibility, the Poroshell 120 EC-C18, 4 μm offers the best place to start your method development. However, if you are working with challenging analytes, the Poroshell 120 family has many other chemistries.



A variety of bonded phases means you never have to compromise on selectivity

Poroshell 120 columns are made at the same facility as the Agilent industry-leading ZORBAX column family. The bonding chemistries used with Poroshell 120 columns mirror those of all ZORBAX columns, giving you the advantages of easier method transfer and assured scalability from lab to lab, around the world.

All the selectivities you need to perfect your separation

An excellent first choice

Poroshell 120 EC-C18 (USP L1)* and EC-C8 (USP L7)*

You can count on this high-performance phase to deliver excellent peak shape and resolution for acids, bases, and neutrals. This chemistry is very similar to the ZORBAX Eclipse Plus phase, for easy method transferability.



TIPS & TOOLS

Watch our video demonstration to learn how to transfer a naproxen method to Poroshell 120 columns, and optimize your LC system for the best results.

Go to www.agilent.com/chem/poroshell120video



High pH applications

Poroshell HPH-C18 (USP L1)* and HPH-C8 (USP L7)*

The silica in this special chemistry has been modified with a proprietary process to increase stability at high pH levels.

Poroshell 120 Phenyl-Hexyl (USP L11)*

This phase offers an alternative selectivity for phenyl groups, and is very similar to ZORBAX Eclipse Plus Phenyl-Hexyl for easy method transfer.

Poroshell 120 PFP (USP L43)*

Provides an alternative selectivity for halogenated compounds and polar analytes.

Poroshell 120 Bonus-RP (USP L60)

Bonus-RP is polar-embedded to improve peak shape for basic compounds at low- and mid-pH. This phase is the same as ZORBAX Bonus-RP.



Low pH applications

StableBond SB-C18 (USP L1) and SB-C8 (USP L7)

StableBond performs well with acids, bases, and neutrals—with superior lifetime at low pH. What's more, these phases transfer readily from ZORBAX SB-C18 and ZORBAX SB-C8 phase chemistries.

Poroshell 120 HILIC*

With its unbonded silica, Poroshell 120 HILIC enables you to retain and separate small polar analytes.

Poroshell 120 EC-CN (USP L10)

Similar to ZORBAX Eclipse XDB-CN, this cyano phase simplifies method transfer.

Poroshell 120 SB-Aq

This proprietary phase provides an alternative selectivity option, and is ideal for polar compounds and high aqueous conditions. Its chemistry is the same as ZORBAX SB-Aq.

*Available in both 2.7 µm and 4 µm particle sizes.

TIPS & TOOLS

Use the Poroshell selectivity poster to find the best column for your small molecule analysis: see *A Family of Phase Choices to Perfect Every Separation* (publication 5991-6240EN).





What makes Poroshell 120 unique? End-to-end quality control

Single-step coacervation: a key to reproducibility

Some manufacturers laboriously create the porous shell by applying layer after layer of particles. At Agilent, however, we apply the porous shell in one single step—similar to the coacervation technique used to make traditional ZORBAX columns. This unique single-step process delivers higher yields and more column-to-column reproducibility.



Uncompromising standards translate into reproducible results

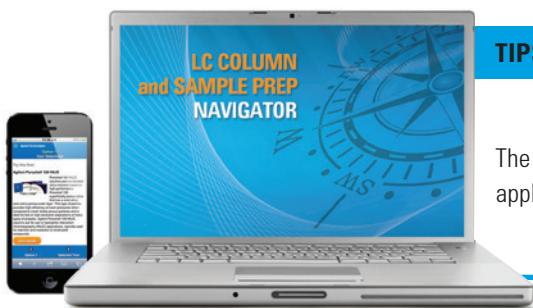
Agilent has more than 40 years of experience manufacturing LC columns. We make all of our ZORBAX and Poroshell 120 columns in our manufacturing location in Newport, Delaware. Our experience has shown that reproducible results can only happen when every column meets the highest industry standards.

That's why we manufacture our own silica and bonded phases—and test our silica more than seven times, including chromatographic tests, across multiple sample types. We also offer multiple particle sizes, chemistries, lengths, and IDs to provide global scalability and transferability.



"Better quality than other coreshell columns."

ACADEMIC LAB



TIPS & TOOLS

The easy way to find the best Agilent LC column or sample prep product for your application. Visit www.navigators.chem.agilent.com

Poroshell 120, 2.7 μ m

| Size (mm) | EC-C18 | EC-C8 | SB-C18 | SB-C8 | HPH-C18 | HPH-C8 | SB-Aq | Bonus-RP | EC-CN | PPF |
|-----------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 4.6 x 150 | 693975-902 | 693975-906 | 683975-902 | 683975-906 | 693975-702 | 693975-706 | 683975-914 | 693968-901 | 693975-905 | 693975-408 |
| 4.6 x 100 | 695975-902 | 695975-906 | 685975-902 | 685975-906 | 695975-702 | 695975-706 | 685975-914 | 695968-901 | 695975-905 | 695975-408 |
| 4.6 x 75 | 697975-902 | 697975-906 | 687975-902 | | | | | | | |
| 4.6 x 50 | 699975-902 | 699975-906 | 689975-902 | 689975-906 | 699975-702 | 699975-706 | 689975-914 | 699968-901 | 699975-905 | 699975-408 |
| 4.6 x 30 | 691975-902 | 691975-906 | 681975-902 | | | | | | | |
| 3.0 x 150 | 693975-302 | 693975-306 | 683975-302 | 683975-306 | 693975-502 | 693975-506 | 683975-314 | 693968-301 | 693975-305 | 693975-308 |
| 3.0 x 100 | 695975-302 | 695975-306 | 685975-302 | 685975-306 | 695975-502 | 695975-506 | 685975-314 | 695968-301 | 695975-305 | 695975-308 |
| 3.0 x 75 | 697975-302 | 697975-306 | 687975-302 | | | | | | | |
| 3.0 x 50 | 699975-302 | 699975-306 | 689975-302 | 689975-306 | 699975-502 | 699975-506 | 689975-314 | 699968-301 | 699975-305 | 699975-308 |
| 3.0 x 30 | 691975-302 | 691975-306 | 681975-302 | | | | | | | |
| 2.1 x 150 | 693775-902 | 693775-906 | 683775-902 | 683775-906 | 693775-702 | 693775-706 | 683775-914 | 693768-901 | 693775-905 | 693775-408 |
| 2.1 x 100 | 695775-902 | 695775-906 | 685775-902 | 685775-906 | 695775-702 | 695775-706 | 685775-914 | 695768-901 | 695775-905 | 695775-408 |
| 2.1 x 75 | 697775-902 | 697775-906 | 687775-902 | | | | | | | |
| 2.1 x 50 | 699775-902 | 699775-906 | 689775-902 | 689775-906 | 699775-702 | 699775-706 | 689775-914 | 699768-901 | 699775-905 | 699775-408 |
| 2.1 x 30 | 691775-902 | 691775-906 | 681775-902 | | | | | | | |

Poroshell 120, 4 μ m

| Size (mm) | EC-C18 | EC-C8 | HPH-C18 | HPH-C8 | PPF | Phenyl-Hexyl | HILIC |
|-----------|------------|------------|------------|------------|------------|--------------|------------|
| 4.6 x 250 | 690970-902 | 690970-906 | 690970-702 | 690970-706 | 690970-408 | 690970-912 | 690970-901 |
| 4.6 x 150 | 693970-902 | 693970-906 | 693970-702 | 693970-706 | 693970-408 | 693970-912 | 693970-901 |
| 4.6 x 100 | 695970-902 | 695970-906 | 695970-702 | 695970-706 | 695970-408 | 695970-912 | 695970-901 |
| 4.6 x 50 | 699970-902 | 699970-906 | 699970-702 | 699970-706 | 699970-408 | 699970-912 | 699970-901 |
| 3.0 x 250 | 690970-302 | 690970-306 | 690970-502 | 690970-506 | 690970-308 | 690970-312 | 690970-301 |
| 3.0 x 150 | 693970-302 | 693970-306 | 693970-502 | 693970-506 | 693970-308 | 693970-312 | 693970-301 |
| 3.0 x 100 | 695970-302 | 695970-306 | 695970-502 | 695970-506 | 695970-308 | 695970-312 | 695970-301 |
| 3.0 x 50 | 699970-302 | 699970-306 | 699970-502 | 699970-506 | 699970-308 | 699970-312 | 699970-301 |
| 2.1 x 250 | 650750-902 | 650750-906 | 690770-702 | 690770-706 | 650750-408 | 650750-912 | 650750-901 |
| 2.1 x 150 | 693770-902 | 693770-906 | 693770-702 | 693770-706 | 693770-408 | 693770-912 | 693770-901 |
| 2.1 x 100 | 695770-902 | 695770-906 | 695770-702 | 695770-706 | 695770-408 | 695770-912 | 695770-901 |
| 2.1 x 50 | 699770-902 | 699770-906 | 699770-702 | 699770-706 | 699770-408 | 699770-912 | 699770-901 |



Poroshell 120 Fast Guards for UHPLC 2.7 µm Columns

| Size (mm) | EC-C18 | EC-C8 | EC-CN | SB-C18 | SB-C8 | HPH-C18 | HPH-C8 | SB-Aq | Bonus-RP | Phenyl-Hexyl | HILIC | PFP |
|-----------|------------|------------|------------|------------|------------|------------|------------|------------|------------|--------------|------------|------------|
| 4.6 x 5 | 820750-911 | 820750-913 | 820750-927 | 820750-912 | 820750-923 | 820750-921 | 820750-922 | 820750-924 | 820750-925 | 820750-914 | 820750-926 | |
| 3.0 x 5 | 823750-911 | 823750-913 | 823750-927 | 823750-912 | 823750-923 | 823750-921 | 823750-922 | 823750-924 | 823750-925 | 823750-914 | 823750-926 | |
| 2.1 x 5 | 821725-911 | 821725-913 | 821725-927 | 821725-912 | 821725-923 | 821725-921 | 821725-922 | 821725-924 | 821725-925 | 821725-914 | 821725-926 | 821725-915 |

Guard Columns for 4 µm

| Size (mm) | EC-C18 | HPH-C18 | HPH-C8 |
|-----------|------------|------------|------------|
| 4.6 x 5 | 820750-916 | 820750-930 | 820750-929 |
| 3.0 x 5 | 823750-916 | 823750-930 | 823750-929 |
| 2.1 x 5 | 821725-916 | 821725-930 | 821725-929 |



PRIMARY STRUCTURE ANALYSIS

What is a biocolumn?

Biochromatography columns, or biocolumns, are liquid chromatography columns used for the separation of biological compounds such as peptides and proteins, oligonucleotides and polynucleotides, and other biomolecules and complexes. Biocolumns are specifically designed for biomolecule analysis with larger pore sizes to accommodate larger molecule sizes. Media are designed to minimize nonspecific binding of analytes for improved recovery. Separation mechanisms are chosen to either retain biological function so that bioactivity is not lost during analysis, or to deliberately denature for primary structure characterization.

Agilent biocolumns provide solutions for all the major characterization techniques required for your biomolecule analysis. These include:

Titer determination and purification: Use unique technology such as AdvanceBio Bio-Monolith Protein A to perform titer determination and cell-line optimization.

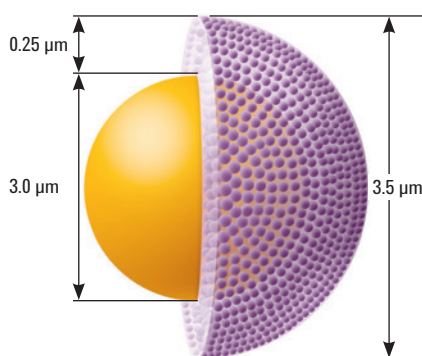
Intact and posttranslational modification: Use key technologies such as AdvanceBio RP-mAb, ZORBAX RRHD 300Å, Poroshell 300, and AdvanceBio Peptide Mapping for confidence in results from primary structural characterization through analysis of intact or fragmented proteins.

Aggregation: Agilent Bio SEC-3 and Agilent Bio SEC-5 accurately measure aggregates (dimers, trimers, tetramers, etc.) and separate low molecular excipients and impurities from larger molecular weight proteins.

Charge variants: Agilent ion-exchange columns include optimized chemistries for monoclonal antibody analysis, such as Agilent Bio mAb and Agilent Bio IEX for accurate isoform analysis.

Glycosylation characterization: Agilent hydrophilic interaction chromatography (HILIC) columns deliver accurate and reproducible glycan and glycopeptide analysis.

Special applications: Includes robust, high efficiency solutions for amino acid analysis through the ZORBAX Eclipse AAA column and AdvanceBio Oligonucleotide columns and standards for synthetic oligonucleotide impurity profiling.



Recently, Agilent introduced the AdvanceBio family to enable protein and mAb characterization. Agilent AdvanceBio columns are designed to advance accuracy and speed for your characterization of monoclonal antibodies and other intact proteins, aggregation with SEC, charge variants with IEX, intact mass, primary structure, and posttranslational modifications (PTMs) by reversed-phase, and cleaved glycan analysis by hydrophilic interaction chromatography.

The AdvanceBio family features the Agilent tradition of innovation in superficially porous silica-based columns for biomolecule separations—which began with Poroshell 300 and now includes AdvanceBio Peptide Mapping, AdvanceBio Glycan Mapping, AdvanceBio RP-mAb, and AdvanceBio Oligonucleotide.

AdvanceBio RP-mAb

- **Improved accuracy:** Superficially porous particles (3.5 μm) with wide pores (450Å) increase mAb resolution while maintaining compatibility with all LC instruments
- **Speed:** Shorter analysis times compared to columns packed with fully porous particles
- **Flexible method development:** Range of chemistries—SB-C8, C4, and Diphenyl
- **Lower costs:** Robust Poroshell packed bed and 2 μm inlet frit extend column lifetime by helping prevent inlet blockage

The only reversed-phase columns focused on the unique challenges of monoclonal antibody characterization

Analysis of intact and reduced monoclonal antibodies are critical measurements for characterizing therapeutic proteins and understanding their efficacy and stability. Poor chromatographic separations can result in rework and even compromise the accuracy of the characterization. Long analysis times negatively impact the throughput of a laboratory and lead to delays in making decisions based on the results of characterization.

To eliminate these problems, Agilent has developed a new reversed-phase column to optimize the performance of intact and reduced mAb analysis. The Agilent AdvanceBio RP-mAb column is based on Poroshell technology with unique engineering for pore size and bonded phases.



TIPS & TOOLS

For more information on the characterization of monoclonal antibody primary structure, see: *Better Characterization of Biomolecules using Agilent AdvanceBio Reversed-Phase Columns* (publication 5991-2032EN)

www.agilent.com/chem/library

Column Specifications

| Bonded Phase | Pore Size | Temp Limits* | pH Range | Endcapped |
|----------------------------|-----------|--------------|----------|-----------|
| AdvanceBio RP-mAb C4 | 450Å | 90 °C | 1.0-8.0 | Yes |
| AdvanceBio RP-mAb SB-C8 | 450Å | 90 °C | 1.0-8.0 | No |
| AdvanceBio RP-mAb Diphenyl | 450Å | 90 °C | 1.0-8.0 | Yes |

Specifications represent typical values only.

*Columns are designed for optimal use at low pH. At pH 6-8, highest column stability for all silica-based columns is obtained by operating at temperatures <40 °C and using low buffer concentrations in the range of 0.01-0.02 M.

High speed, high resolution separation of Herceptin Variant IgG1

Column: AdvanceBio RP-mAb C4
795775-904
2.1 x 100 mm, 3.5 µm

Mobile Phase: A: 0.1% TFA in water:IPA (98:2)
 B: IPA:ACN:Mobile phase A (70:20:10)

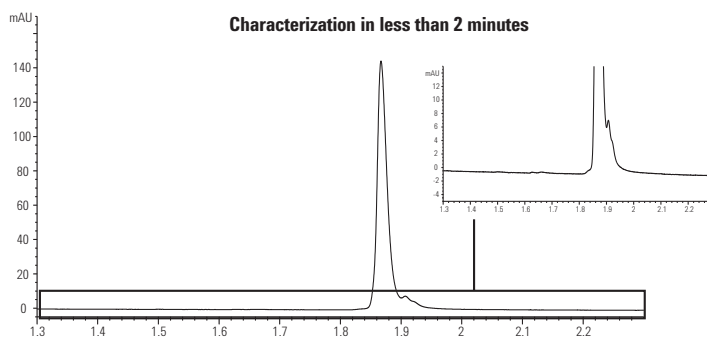
Flow Rate: 1.0 mL/min

Gradient: 10-58% B in 4 min, 1 min wash at 95% B,
 1 min re-equilibration at 10% B

Temperature: 80 °C

Detector: UV, 254 nm

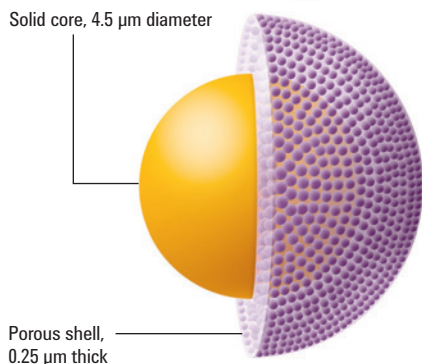
Sample: 5 µL injection of humanized recombinant
 Herceptin Variant IgG1 intact from Creative Biolabs (1 mg/mL)



AdvanceBio RP-mAb C4 provides a sharp peak and resolves fine detail in less than 2 minutes.

AdvanceBio RP-mAb

| Size (mm) | Particle Size (µm) | AdvanceBio RP-mAb C4 USP L26 | AdvanceBio RP-mAb SB-C8 USP L7 | AdvanceBio RP-mAb Diphenyl USP L11 |
|-----------|--------------------|---------------------------------|-----------------------------------|---------------------------------------|
| 4.6 x 150 | 3.5 | 793975-904 | 783975-906 | 793975-944 |
| 4.6 x 100 | 3.5 | 795975-904 | 785975-906 | 795975-944 |
| 4.6 x 50 | 3.5 | 799975-904 | 789975-906 | 799975-944 |
| 2.1 x 150 | 3.5 | 793775-904 | 783775-906 | 793775-944 |
| 2.1 x 100 | 3.5 | 795775-904 | 785775-906 | 795775-944 |
| 2.1 x 75 | 3.5 | 797775-904 | 787775-906 | 797775-944 |
| 2.1 x 50 | 3.5 | 799775-904 | 789775-906 | 799775-944 |



Poroshell 300

- High speed separations of biomolecules with superficially porous particles
- 300Å pores provide high efficiency and recovery with proteins (up to 1,000 kDa)
- Achieve long lifetime at low pH with Poroshell 300SB; at high pH with 300Extend-C18
- Optimize recovery and selectivity with four different bonded phases—300SB-C18, 300SB-C8, 300SB-C3, and 300Extend-C18

Agilent Poroshell 300 columns are ideal for fast separations of proteins and peptides because the 5 μm diameter superficially porous particle allows for fast flow rates to be used while maintaining sharp, efficient peaks. Poroshell columns with StableBond bonded phases provide excellent stability and selectivity choices with TFA and formic acid mobile phases. The Poroshell 300Extend-C18 column can be used from pH 2 to 11 for unique separations. These columns can also be used for analytical protein separations and LC/MS separations. Peptides and proteins are typically separated slowly to reduce the potential peak broadening of these slow diffusing analytes. However, Poroshell columns use a superficially porous particle made with a thin layer of porous silica, 0.25 μm thick, on a solid core of silica. This reduces the diffusion distance for proteins, making rapid HPLC separations of peptides and proteins up to 500 to 1,000 kDa possible with 400/600 bar HPLC systems.



Poroshell 300 Columns

UHPLC Column Specifications

| Bonded Phase | Pore Size | Temp Limits* | pH Range | Endcapped |
|-----------------------------|-----------|--------------------------------------|----------|-----------|
| Poroshell 300SB-C18, C8, C3 | 300Å | 90 °C | 1.0-8.0 | No |
| Poroshell 300Extend-C18 | 300Å | 40 °C above pH 8 60 °C below pH 8 | 2.0-11.0 | Yes |

Specifications represent typical values only

*300StableBond columns are designed for optimal use at low pH. At pH 6 to 8, the highest column stability for all silica-based columns is obtained by operating at temperatures <40 °C and using low buffer concentrations in the range of 0.01 to 0.02 M. At mid or high pH, 300Extend-C18 is recommended.

Poroshell 300 columns separate proteins and peptides in seconds

Column: Poroshell 300SB-C18
660750-902
2.1 x 75 mm, 5 µm

Mobile Phase: A: 0.1% TFA in H₂O
B: 0.07% TFA in ACN

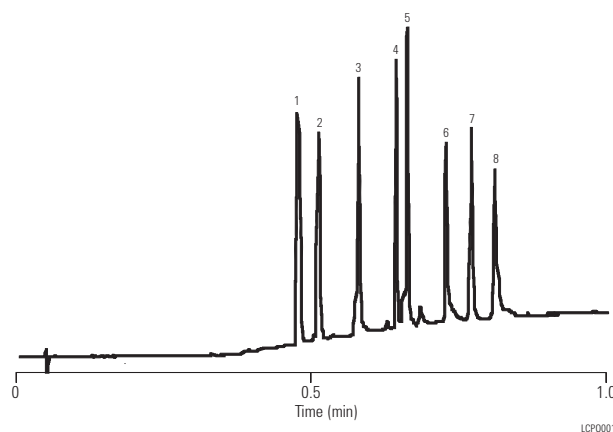
Flow Rate: 3.0 mL/min

Gradient: 5-100% B in 1.0 min

Temperature: 70 °C, 260 bar

Detector: UV, 215 nm

Sample: Proteins and peptides



1. Angiotensin II
2. Neurotensin
3. RNase
4. Insulin
5. Lysozyme
6. Myoglobin
7. Carbonic anhydrase
8. Ovalbumin

This separation of eight polypeptides and proteins is completed in less than 60 seconds. Each peak is sharp and efficient.

Poroshell 300

| Hardware | Description | Size (mm) | Particle Size (µm) | Poroshell 300SB-C18 | Poroshell 300SB-C8 | Poroshell 300SB-C3 | Poroshell 300Extend-C18 |
|----------|-----------------------|------------|--------------------|---------------------|--------------------|--------------------|-------------------------|
| | Narrow Bore | 2.1 x 75 | 5 | 660750-902 | 660750-906 | 660750-909 | 670750-902 |
| | MicroBore | 1.0 x 75 | 5 | 661750-902 | 661750-906 | 661750-909 | 671750-902 |
| | Capillary | 0.5 x 75 | 5 | | 5065-4468 | | |
| ZGC | Guard Cartridge, 4/pk | 2.1 x 12.5 | 5 | 821075-920 | 821075-918 | 821075-924 | |
| ZGC | Guard Hardware Kit | | | 820999-901 | 820999-901 | 820999-901 | |
| | MicroBore Guard, 3/pk | 1.0 x 17 | 5 | 5185-5968 | 5185-5968 | 5185-5968 | 5185-5968 |



AdvanceBio Peptide Mapping

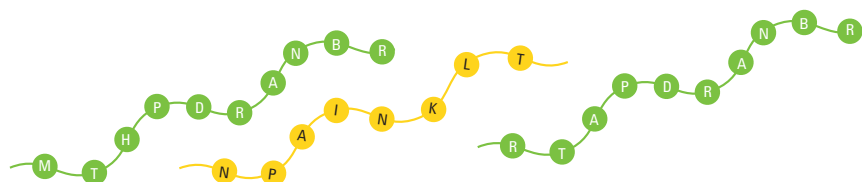
- Greater analytical confidence: Each batch of AdvanceBio Peptide Mapping media is tested with a rigorous peptide mix to ensure suitability and reproducibility, and to enable the identification of key peptides in complex peptide maps
- Save time: 2 to 3 times faster than fully porous HPLC columns
- Every instrument works harder: 4.6, 3.0, and 2.1 mm id columns are stable to 600 bar, and deliver excellent performance on 400 bar systems.
- Increased flexibility: Achieve increased MS sensitivity with formic acid mobile phases on any HPLC

These advanced biocolumns feature a 120Å pore size with superficially porous 2.7 µm particles. They are specially tested with a challenging peptides mix to ensure reliable peptide mapping performance. In addition, AdvanceBio Peptide Mapping columns deliver excellent results for conventional HPLC instruments such as Waters Alliance.

Column Specifications

| Bonded Phase | Pore Size | Temp Limits | pH Range | Endcapped |
|--------------|-----------|-------------|----------|-----------|
| EC-C18 | 120Å | 60 °C | 2.0-8.0 | Double |

Specifications represent typical values only



TIPS & TOOLS

Do you want to know how scientists are using the AdvanceBio Peptide Mapping column?
 Refer to: Amano, M. *et al.* Detection of Histidine Oxidation in a Monoclonal Immunoglobulin Gamma (Igg) 1 Antibody. *Analytical Chemistry*, 2014, 86 (15): 7536-7543.

Leah G. Luna and Katherine Coady. Identification of *X. laevis* Vitellogenin Peptide Biomarkers for Quantification by Liquid Chromatography Tandem Mass Spectrometry. *J. Anal Bioanal Tech*, 2014, 5:3.

AdvanceBio Peptide Mapping

| Description | Part No. |
|---------------------------|------------|
| 4.6 x 150 mm, 2.7 μ m | 653950-902 |
| 3.0 x 150 mm, 2.7 μ m | 653950-302 |
| 2.1 x 250 mm, 2.7 μ m | 651750-902 |
| 2.1 x 150 mm, 2.7 μ m | 653750-902 |
| 2.1 x 100 mm, 2.7 μ m | 655750-902 |
| 4.6 x 5 mm, Fast Guard* | 850750-911 |
| 3.0 x 5 mm, Fast Guard* | 853750-911 |
| 2.1 x 5 mm, Fast Guard* | 851725-911 |

*Fast guards extend column lifetime without slowing down the separation or affecting resolution.

**Agilent Peptide Quality Control Standard**

Use the Agilent ten-peptide quality control standard, the same standard Agilent uses to QC its columns, to evaluate your column performance over its lifetime. It can be used for HPLC or LC/MS. Approximately 20 injections per vial.

Agilent Peptide Quality Control Standard

| Description | Part No. |
|---|-----------|
| Peptide quality control standard, 71 μ g in 2 mL vial | 5190-0583 |

TIPS & TOOLS

Peptide mapping is a powerful technique and the most widely used identity test for proteins, particularly those produced by recombinant means. There are several considerations to be made in addition to column selection for reproducible and accurate peptide maps, including protein digestion, sample prep, method optimization, and so on. For fundamental techniques used for peptide mapping procedures and emphasized considerations for optimizing your peptide mapping separations to achieve the best possible results, see: *Keys for Enabling Optimum Peptide Characterizations: A Peptide Mapping "How to" Guide* (publication 5991-2348EN)

www.agilent.com/chem/library



GLYCOSYLATION CHARACTERIZATION

Posttranslational modifications to the primary amino acid sequence, including glycosylation, have functional consequences and can impact efficacy and immunogenicity of a biopharmaceutical. The structure of the glycan also contributes to the half-life of the protein in plasma and the ability of the monoclonal antibody to trigger the immune response required for efficacy. Regulatory authorities consider glycosylation to be one of the critical quality attributes and, therefore, it must be characterized and quantified, with acceptable ranges determined, as part of the development process for a glycoprotein innovator, biosimilar, or biobetter pharmaceutical.

There are a number of analytical methods used to obtain information about the structure and form of protein glycosylation.

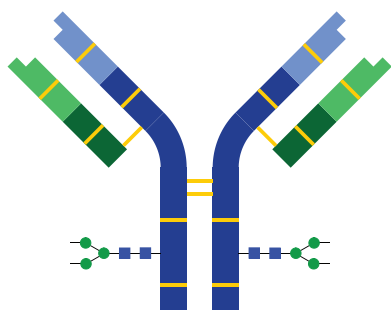
- For structural identification, including identification of glycosylation sites, mass spec detection is used with reversed-phase and hydrophilic interaction liquid chromatography (HILIC).
- Glycans containing sialic acid will also impart additional charge to the protein and can be characterized by ion-exchange chromatography.

Having characterized the glycoprotein and glycopeptide fragments to obtain information about the number and position of the glycosylation sites, it is then necessary to identify and quantify the individual glycans. To do this the glycans must be cleaved from the protein and analyzed using HILIC columns. As glycans have no chromophore, derivatization with a fluorophore is carried out to enable FLD detection to map and quantify the glycans.

Hydrophilic Interaction Column Selection

| Application | Agilent Columns | Notes |
|---|---------------------------|---|
| Glycans cleaved from a glycoprotein including monoclonal antibodies | AdvanceBio Glycan Mapping | Amide bonded phase for rapid equilibration and enhanced selectivity for glycans |
| | 2.7 μm | Based on Poroshell technology to give a superficially porous particle with reduced diffusion distances to give high resolution separations at lower pressures and enable the use of longer column lengths for increased separation efficiency |
| Hydrophilic peptides and glycopeptides | AdvanceBio Glycan Mapping | The amide bonded phase provides an alternative HILIC functionality for small hydrophilic and glycopeptides |





N-Glycan Analysis

AdvanceBio Glycan Mapping columns, standards, and sample preparation products for the selective removal of the N-glycans from a glycoprotein, including monoclonal antibodies.

- **Resolution**—high resolution separations are achieved using 2.7 μm particles packed in the 250 mm column. This increased resolution enables accurate quantitation of target glycans and changes to the protein glycosylation profile that may have occurred during expression.
- **Comprehensive methods**—for sample preparation, chromatographic analysis, and data interpretations to ensure reproducibility, and accuracy of identification and quantitation.
- **Simplicity of ordering**—a single part number to order the full sample preparation workflow, for protein solubilization to purification, of the 2-AB labeled glycans, plus kits for each part of the sample preparation workflow for versatility.

Column Specifications

| Bonded Phase | ID (mm) | Particle Size (μm) | Endcapped | pH Stability | Operating Temperature | Pressure Limit |
|--------------|-------------|---------------------------------|-----------|--------------|-----------------------|----------------|
| Amide HILIC | 2.1 and 4.6 | 2.7, superficially porous | No | 2-7 | 60 °C | 600 bar |

The mapping of the N-linked glycan component of a glycoprotein, including monoclonal antibodies, requires the N-glycans to be enzymatically cleaved, using PNGase F, from the protein amino acid backbone. The cleaved N-glycans can be analyzed by hydrophilic interaction chromatography with MS detection, or after derivatization with a fluorophore, 2-aminobenzamide (2-AB), analyzed using HPLC/UHPLC using either FLD or MS.

OLIGONUCLEOTIDES

The need for resolution and lifetime

Successful ion-pair reversed-phase separation of the trityl-off, deprotected oligos requires columns that have high resolving power and are robust enough to withstand the relatively aggressive analysis conditions.

Without sufficient resolution, the accuracy and precision of measurements can be compromised, leading to a lack in confidence in the analytical results.

Columns that are not robust will have a short lifetime, resulting in frequent replacement with the related disruption to workflows and increased costs.

Agilent AdvanceBio Oligonucleotide columns feature high efficiency, 2.7 μm superficially porous Poroshell particles. The particles are chemically modified using proprietary technology that makes them very resistant to high pH mobile phases. They are bonded with an endcapped C18 phase that delivers excellent selectivity for oligonucleotides.

To ensure performance for your separations every batch of AdvanceBio Oligonucleotide media is tested with an Agilent Oligonucleotide Resolution standard.

Poroshell 2.7 μm particles with high pH resistance and C18 endcapping provide high-resolution separations of oligonucleotides with long column lifetime on HPLC and UHPLC systems to improve reliability of results and reduce costs.

Column Specifications

| Bonded Phase | Pore Size | Temp Limits | pH Range | Endcapped |
|--------------|-----------|-------------|----------|-----------|
| C18 | 100Å | 65 °C | 3.0-11.0 | Double |

Agilent innovation: The first high-pH stable superficially porous particle based LC column for oligonucleotide analysis

Oligonucleotide separations using triethylammonium acetate (TEAA)

Unmodified silica particles are prone to dissolution in basic mobile phases, leading to reduced column lifetimes. AdvanceBio Oligonucleotide columns have excellent stability with a high pH TEAA-containing mobile phase.

**Column: AdvanceBio Oligonucleotide
659750-702
2.1 x 50 mm**

Mobile Phase: A: 100 mM TEAA in water
B: 100 mM TEAA in acetonitrile

Flow Rate: 0.69 mL/min

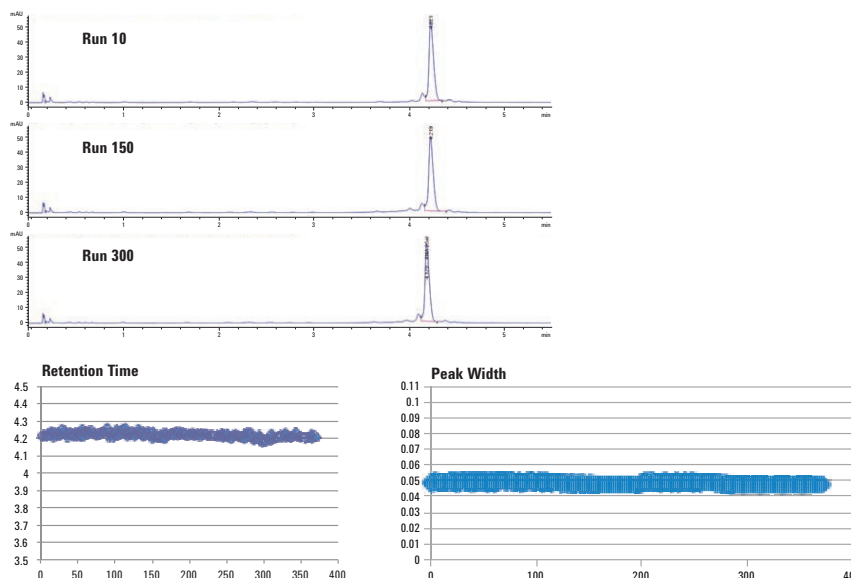
Gradient: 7% B to 11% B in 5 min
11% B to 80% B in 5.01 min
Hold at 80% B for 5.50 min
80% B to 7% B in 5.56 min
Total run time 8.5 min

Temperature: 65 °C

Detector: UV, 260 nm

Sample: 25 mer DNA

Injection: 1 µL of 0.5 mg/mL



The ability to resolve oligonucleotides that differ by a single nucleotide is important for accurate characterization. The AdvanceBio Oligonucleotide column resolves N and N-1 for the Agilent Oligonucleotide Resolution Standard (14, 17, 20, and 21 mer).

**Column: AdvanceBio Oligonucleotide
659750-702
2.1 x 50 mm**

Mobile Phase: A: 100 mM TEAA in water
B: 100 mM TEAA in acetonitrile

Flow Rate: 0.6 mL/min

Stop Time: 13 min

Post Run: 5 min

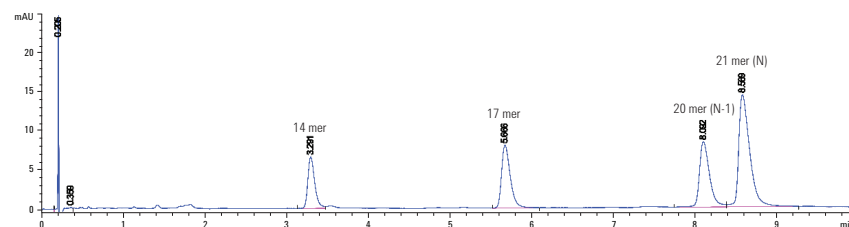
Gradient: 6 to 8% B in 12 min

Temperature: 65 °C

Detector: UV, 260 nm

Sample: Agilent Oligonucleotide Resolution
Standard (p/n 5190-9028)

Injection: 0.5 µL



Assured Performance

Agilent also offers an Oligonucleotide Ladder standard contains 15, 20, 25, 30, 35, and 40 mer synthetic oligodeoxythymidines, an excellent tool for demonstrating column selectivity and reproducibility.

**Column: AdvanceBio Oligonucleotide
659750-702
2.1 x 50 mm**

Mobile Phase: A: 100 mM TEAA in water
B: 100 mM TEAA in acetonitrile

Flow Rate: 0.6 mL/min

Gradient: 6 to 8% B in 12 min

Stop Time: 13 min

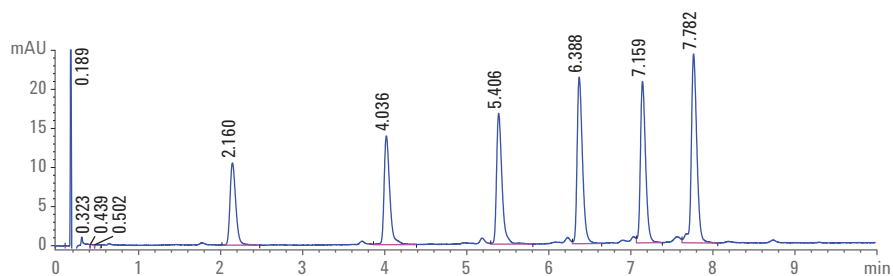
Post Run: 5 min

Temperature: 65 °C

Detector: UV, 260 nm

Sample: Agilent Oligonucleotide Resolution
Standard (p/n 5190-9028)

Injection: 0.5 µL



Agilent Solutions for Oligonucleotides

Oligonucleotide Purification

Agilent PLRP-S and PL-SAX

For the purification of oligonucleotides, chemically and thermally stable polymeric HPLC media is required to achieve high purity with acceptable column lifetimes. There is no deterioration in column performance when PLRP-S columns are used at 80 °C with ion pairing agents, including TEA, for separating trityl-on from trityl-off oligos. PL-SAX 1000Å columns separate deprotected oligos under denaturing high pH conditions. The quaternary amine functionality on the polymeric particles enables ion-exchange separations at high pH, improving chromatography for self-complementary sequences.

TOP-DNA and TOP-RNA purification cartridges

Agilent TOP-DNA and TOP-RNA deliver outstanding yields of high purity synthetic DNA and RNA oligonucleotides by removing

interfering salts, incomplete synthesis products and other impurities in a few simple steps. Perform detritylation of both DNA and RNA oligos in the cartridge. The 96-well format ensures that purification does not limit the throughput.

Nucleic Acid Solutions

Flexible Therapeutic Oligo Manufacturing and Development Services

The Agilent Nucleic Acid Solutions Division offers industry-leading experience to efficiently advance your lead oligo candidates from the clinic to market with a common goal of patient health and safety. Agilent has experience with all classes of oligo APIs, and supports customers from toxicology through commercialization. The Agilent GMP facility, located in Boulder, Colorado houses a broad range of synthesis and purification equipment for production of grams for toxicology and clinical research use, to tens of kilograms for late-stage clinical trials as well as commercialization.

Agilent AdvanceBio Oligonucleotide Columns and Standards

| Description | Part No. |
|-------------------------------------|------------|
| Columns | |
| 2.1 x 50 mm, 2.7 µm | 659750-702 |
| 2.1 x 100 mm, 2.7 µm | 655750-702 |
| 2.1 x 150 mm, 2.7 µm | 653750-702 |
| 2.1 mm Fast Guard | 821725-921 |
| 4.6 x 50 mm, 2.7 µm | 659950-702 |
| 4.6 x 100 mm, 2.7 µm | 655950-702 |
| 4.6 x 150 mm, 2.7 µm | 653950-702 |
| 4.6 mm Fast Guard | 820750-921 |
| Standards | |
| Oligonucleotide Resolution Standard | 5190-9028 |
| Oligonucleotide Ladder Standard | 5190-9029 |

CHARGE VARIANT ANALYSIS



Agilent Bio mAb HPLC Columns

- A packing support composed of a rigid, spherical, highly cross-linked polystyrene divinylbenzene (PS/DVB) nonporous bead
- Particles grafted with a hydrophilic polymeric layer, virtually eliminating nonspecific binding of antibody proteins
- A different process is used to layer the weak cation-exchange phase to the particle, giving it a higher density than the Agilent Bio WCX column particles
- Specifically designed for the separation of charge isoforms of monoclonal antibodies

Thorough characterization of monoclonal antibodies includes the identification and monitoring of acidic and basic isoforms. Agilent Bio mAb HPLC columns feature a unique resin specifically designed for high resolution, charge-based separations of monoclonal antibodies. These columns are compatible with aqueous solution buffers, acetonitrile/acetone/methanol, and water mixtures. Commonly used buffers are phosphate, tris, MES, and acetate.

Bio mAb columns are available in 1.7, 3, 5, and 10 μm particle sizes, providing higher resolution with smaller particles.

Column Specifications

| Bonded Phase | ID | Particle Size | pH Stability | Operating Temperature Limit | Flow Rate |
|------------------------------------|----------------|---------------------------------|--------------|-----------------------------|----------------|
| Weak cation-exchange (carboxylate) | 2.1 and 4.6 mm | 1.7, 3, 5, and 10 μm | 2-12 | 80 °C | 0.1-1.0 mL/min |



TIPS & TOOLS

Are you looking to increase your throughput for charge variant analysis of monoclonal antibodies? If so, see:

Reducing Cycle Time for Charge Variant Analysis of Monoclonal Antibodies Alternating Column Regeneration Using an Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port Valve (publication 5991-4722EN)

www.agilent.com/chem/library

Virtually eliminate retention time variations

Column: Bio mAb, stainless steel
5190-2413
4.6 x 250 mm, 10 µm

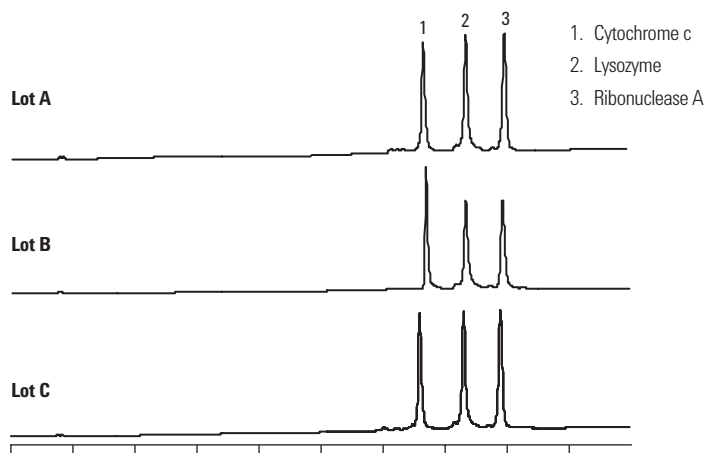
Mobile Phase: A: 10 mM sodium phosphate, pH 6.0
 B: A + 1.0 M sodium chloride

Flow Rate: 1.0 mL/min

Gradient: 0-100% B in 42 min

Temperature: 25 °C

Detector: UV, 214 nm



The combination of well-controlled resin production, column surface chemistry, and column packing virtually eliminates retention time variations from column-to-column and lot-to-lot.

Agilent Bio mAb HPLC Columns

| Size (mm) | Particle Size (µm) | Bio mAb PEEK | Pressure Limit | Bio mAb Stainless Steel | Pressure Limit |
|-----------------|--------------------|--------------|-------------------|-------------------------|-------------------|
| 21.2 x 250 | 5 | | | 5190-6885 | 275 bar, 4000 psi |
| 10 x 250 | 5 | | | 5190-6884 | 275 bar, 4000 psi |
| 4.6 x 250 | 10 | 5190-2415 | 275 bar, 4000 psi | 5190-2413 | 275 bar, 4000 psi |
| 4.6 x 50 | 10 | 5190-2416 | 275 bar, 4000 psi | | |
| 4.6 x 250 | 5 | 5190-2407 | 400 bar, 5800 psi | 5190-2405 | 400 bar, 5800 psi |
| 4.6 x 50 | 5 | 5190-2408 | 400 bar, 5800 psi | | |
| 4.6 x 50 | 3 | | | 5190-2403 | 551 bar, 8000 psi |
| 4.6 x 50 | 1.7 | | | 5190-2401 | 600 bar, 8700 psi |
| 4.0 x 10, Guard | 10 | | | 5190-2414 | 275 bar, 4000 psi |
| 4.0 x 10, Guard | 5 | | | 5190-2406 | 413 bar, 6000 psi |
| 4.0 x 10, Guard | 3 | | | 5190-2404 | 551 bar, 8000 psi |
| 4.0 x 10, Guard | 1.7 | | | 5190-2402 | 600 bar, 8700 psi |
| 2.1 x 250 | 10 | 5190-2419 | 275 bar, 4000 psi | | |
| 2.1 x 50 | 10 | 5190-2420 | 275 bar, 4000 psi | | |
| 2.1 x 250 | 5 | 5190-2411 | 400 bar, 5800 psi | | |
| 2.1 x 50 | 5 | 5190-2412 | 400 bar, 5800 psi | | |



Agilent Bio IEX HPLC Columns

- Highly cross-linked and rigid nonporous poly(styrene divinylbenzene) (PS/DVB) particles are grafted with a hydrophilic polymeric layer, eliminating nonspecific binding
- Uniform, densely packed ion-exchange functional groups are chemically bonded to the hydrophilic layer (multiple ion-exchange groups per anchor) to increase column capacity
- Particles, coating, and bonding are resistant to high pressures, promoting higher resolution and faster separations
- Multiple ion-exchange groups are captured on one anchor to increase capacity

Agilent Bio IEX HPLC columns are packed with polymeric, nonporous, ion-exchange particles and are designed for high resolution, high recovery, and highly efficient separations of peptides, oligonucleotides, and proteins.

The Bio IEX family includes strong cation-exchange (SCX), weak cation-exchange (WCX), strong anion-exchange (SAX), and weak anion-exchange (WAX) phases. All phases are available in 1.7, 3, 5, and 10 μm nonporous particles.

Column Specifications

| Bonded Phase | ID (mm) | Particle Size (μm) | pH Stability | Operating Temperature Limit | Flow Rate |
|---|-------------|---------------------------------|--------------|-----------------------------|----------------|
| SCX (strong cation-exchange)— SO_3H | 2.1 and 4.6 | 1.7, 3, 5, and 10 | 2-12 | 80 °C | 0.1-1.0 mL/min |
| WCX (weak cation-exchange)— COOH | | | | | |
| SAX (strong anion-exchange)— $\text{N}(\text{CH}_3)_3$ | | | | | |
| WAX (weak anion-exchange)— $\text{N}(\text{C}_2\text{H}_5)_2$ | | | | | |

TIPS & TOOLS

For further information on optimizing your charge variant analysis, see:

Ion-exchange chromatography for biomolecule analysis: a "how-to" guide (publication 5991-3775EN), and

Agilent ion-exchange BioHPLC columns, characterize charged variants of proteins with speed and confidence (publication 5991-2449EN)

www.agilent.com/chem/library



PL-SAX Strong Anion-Exchange Columns

- Small particles deliver excellent chromatographic performance
- Wide range of particle sizes and two pore sizes for flexible analysis to scale up purification
- Exceptional stability for long column lifetime

PL-SAX $-N(CH_3)_3^+$ is ideal for the anion-exchange HPLC separations of proteins, peptides, and deprotected synthetic oligonucleotides under denaturing conditions. The strong anion-exchange functionality, covalently linked to a chemically stable fully porous polymer, extends the operating pH range. In addition, the anion-exchange capacity is independent of pH. For synthetic oligonucleotides, separations using denaturing conditions of temperature, organic solvent, and high pH are all possible. PL-SAX delivers improved chromatography for self-complementary or G-rich sequences that may associate to form aggregates or hairpin structures. The 5 μm material provides high efficiency separations of n and n-1 sequences. A wide range of particle sizes and column geometries permits analysis scale-up to purification. The strong anion-exchange functionality provides a material with exceptional chemical and thermal stability, even with sodium hydroxide eluents, leading to long column lifetime.

Column Specifications

| Bonded Phase | ID | Particle Size | Pore Size | pH Stability | Operating Temperature Limit |
|-----------------------|--------------------------------------|---------------------|--------------------|--------------|-----------------------------|
| Strong anion-exchange | 2.1, 4.6, 7.5, 25, 50, and 100 | 5, 8, 10, and 30 | 1000Å and 4000Å | 1-14 | 80 °C |



AGGREGATION ANALYSIS

Agilent Bio SEC-3 Columns

Agilent Bio SEC-3 columns offer speed and resolution advantages over other SEC columns, thanks to their small, efficient particles.

- Faster separations than large-particle SEC columns
- High resolution: Sharper peaks and better protein recovery
- Exceptional loading capacity and recovery due to proprietary hydrophilic layer
- Flexible method development: Compatible with most aqueous buffers
- Excellent stability under both high- and low-salt conditions
- Reliable, consistent performance: narrowly dispersed particles; proprietary hydrophilic layer provides for minimal secondary interactions
- Robust particles compatible with multi-detectors including light scattering

TIPS & TOOLS

Deactivated/silanized vials have inert surfaces that will not interact with metals, biologicals, or proteins, and will not cause pH shifts. Avoid standard polypropylene vials for biological or light-sensitive compounds.

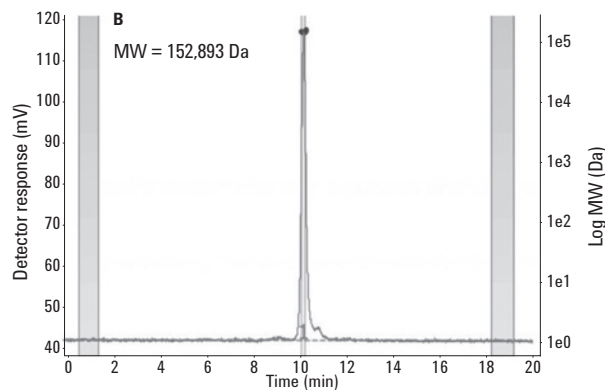
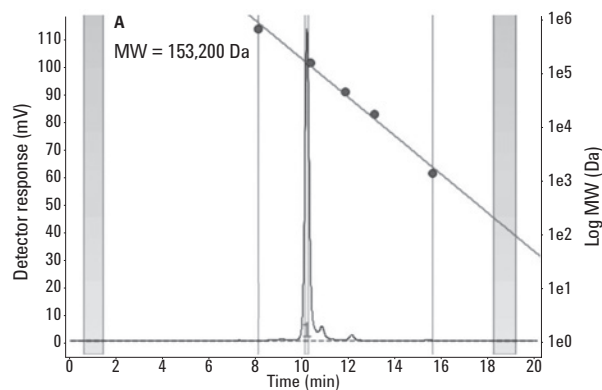
With higher resolution for faster peptide and protein separations, Bio SEC-3 columns help you achieve more consistent SEC separations. Each column is packed with spherical, narrowly dispersed 3 μm silica particles coated with a proprietary hydrophilic layer for high recovery and minimal secondary interactions, which provides more consistent separations. This thin polymeric layer is chemically bonded to pure, mechanically stable silica under controlled conditions, ensuring a highly efficient and stable size exclusion particle.

Column Specifications

| Pore Size | Particle Size (μm) | MW Range | pH Range | Max Pressure | Flow Rate |
|-----------|---------------------------------|-----------------|----------|-------------------|------------------------------|
| 100Å | 3 | 100-100,000 | 2-8.5 | 137 bar, 2000 psi | 1.0-10.0 mL/min (21.2 mm id) |
| | | | | | 0.2-1.2 mL/min (7.8 mm id) |
| | | | | | 0.1-0.4 mL/min (4.6 mm id) |
| 150Å | 3 | 500-150,000 | 2-8.5 | 137 bar, 2000 psi | 1.0-10.0 mL/min (21.2 mm id) |
| | | | | | 0.2-1.2 mL/min (7.8 mm id) |
| | | | | | 0.1-0.4 mL/min (4.6 mm id) |
| 300Å | 3 | 5,000-1,250,000 | 2-8.5 | 137 bar, 2000 psi | 1.0-10.0 mL/min (21.2 mm id) |
| | | | | | 0.2-1.2 mL/min (7.8 mm id) |
| | | | | | 0.1-0.4 mL/min (4.6 mm id) |

Comparison of traditional SEC analysis

Column: **Bio SEC-3, 300Å**
5190-2511
7.8 x 300 mm, 3 μm
 Mobile Phase: PBS, pH 7.4
 Flow Rate: 0.75 mL/min



Comparison of traditional SEC analysis with UV detection and column calibration (A) and LS analysis at 90° (B) for anti-DYKDDDDK. Both MW results lie in the same range of approximately 153,000 Da.

Advance Bio SEC-3

| Size (mm) | Particle Size (μm) | Bio SEC-3 100Å USP L33 | Bio SEC-3 150Å USP L33 | Bio SEC-3 300Å USP L33 |
|------------------|--------------------|------------------------|------------------------|------------------------|
| 21.2 x 300 | 3 | 5190-6850 | 5190-6851 | 5190-6852 |
| 21.2 x 50, Guard | 3 | 5190-6854 | 5190-6855 | 5190-6856 |
| 7.8 x 300 | 3 | 5190-2501 | 5190-2506 | 5190-2511 |
| 7.8 x 150 | 3 | 5190-2502 | 5190-2507 | 5190-2512 |
| 7.8 x 50, Guard | 3 | 5190-2505 | 5190-2510 | 5190-2515 |
| 4.6 x 300 | 3 | 5190-2503 | 5190-2508 | 5190-2513 |
| 4.6 x 150 | 3 | 5190-2504 | 5190-2509 | 5190-2514 |
| 4.6 x 50, Guard | 3 | 5190-6846 | 5190-6847 | 5190-6848 |

TIPS & TOOLS

To further understand molecular weight determination and aggregation analysis using Agilent BioSEC-3 columns, see:
Agilent BioHPLC Size Exclusion Chromatography Columns brochure (publication 5991-2898EN).

www.agilent.com/chem/library



Agilent Bio SEC-5 Columns

- Exceptional resolution for large molecules
- High stability and efficiency due to a proprietary neutral hydrophilic layer
- Improved peak capacity and resolution due to specially designed packing that increases pore volume
- Rugged performance: outstanding reproducibility and column lifetime
- Excellent stability, even under high-pH, high-salt, and low-salt conditions
- Flexible method development: Compatible with most aqueous buffers
- Broad applicability: Up to 2000Å pore size for vaccines and high molecular weight biomolecules

TIPS & TOOLS

Deactivated/silanized vials have inert surfaces that will not interact with metals, biologicals or proteins, and will not cause pH shifts. Avoid standard polypropylene vials for biological or light-sensitive compounds.

For large biomolecules and samples with components of multiple molecular weights, Agilent Bio SEC-5 columns are an ideal choice. They are packed with 5 µm silica particles coated with a proprietary, neutral, hydrophilic layer for maximum efficiency and stability, with six different pore sizes to provide optimum resolution over the molecular weight range.

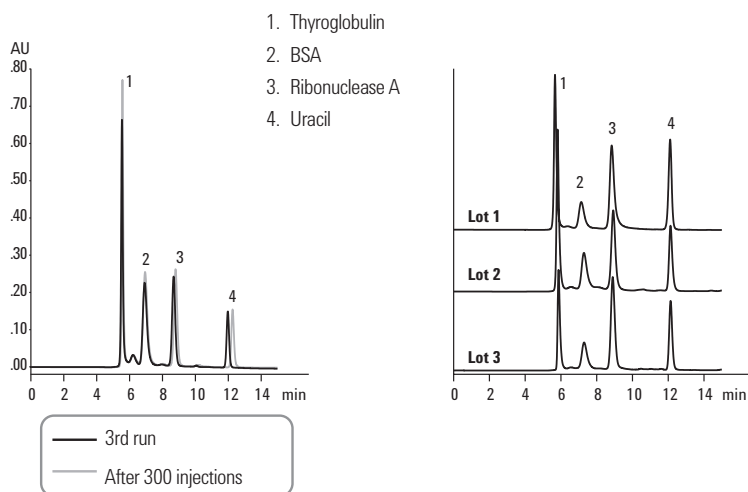
Column Specifications

| Pore Size | Particle Size (µm) | MW Range | pH Range | Max Pressure | Flow Rate |
|-----------|--------------------|------------------|----------|-------------------|------------------------------|
| 100Å | 5 | 100-100,000 | 2-8.5 | 137 bar, 2000 psi | 1.0-10.0 mL/min (21.2 mm id) |
| | | | | | 0.2-1.2 mL/min (7.8 mm id) |
| | | | | | 0.1-0.4 mL/min (4.6 mm id) |
| 150Å | 5 | 500-150,000 | 2-8.5 | 137 bar, 2000 psi | 1.0-10.0 mL/min (21.2 mm id) |
| | | | | | 0.2-1.2 mL/min (7.8 mm id) |
| | | | | | 0.1-0.4 mL/min (4.6 mm id) |
| 300Å | 5 | 5,000-1,250,000 | 2-8.5 | 137 bar, 2000 psi | 1.0-10.0 mL/min (21.2 mm id) |
| | | | | | 0.2-1.2 mL/min (7.8 mm id) |
| | | | | | 0.1-0.4 mL/min (4.6 mm id) |
| 500Å | 5 | 15,000-5,000,000 | 2-8.5 | 137 bar, 2000 psi | 1.0-10.0 mL/min (21.2 mm id) |
| | | | | | 0.2-1.2 mL/min (7.8 mm id) |
| | | | | | 0.1-0.4 mL/min (4.6 mm id) |
| 1000Å | 5 | 50,000-7,500,000 | 2-8.5 | 137 bar, 2000 psi | 1.0-10.0 mL/min (21.2 mm id) |
| | | | | | 0.2-1.2 mL/min (7.8 mm id) |
| | | | | | 0.1-0.4 mL/min (4.6 mm id) |
| 2000Å | 5 | >10,000,000 | 2-8.5 | 137 bar, 2000 psi | 1.0-10.0 mL/min (21.2 mm id) |
| | | | | | 0.2-1.2 mL/min (7.8 mm id) |
| | | | | | 0.1-0.4 mL/min (4.6 mm id) |

Exceptional lot-to-lot reproducibility

Column: Bio SEC-5, 150Å
 5190-2521
 7.8 x 300 mm, 5 µm

Mobile Phase: Sodium phosphate 150 mM, pH 7.0



The four-protein mixture shows excellent retention time reproducibility over 300 injections and on three columns from different manufacturing lots.

Advance Bio SEC-5

| Size (mm) | Particle Size (µm) | Bio SEC-5 100Å USP L33 | Bio SEC-5 150Å USP L33 | Bio SEC-5 300Å USP L33 | Bio SEC-5 500Å USP L33 | Bio SEC-5 1000Å USP L33 | Bio SEC-5 2000Å USP L33 |
|------------------|--------------------|------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|
| 21.2 x 300 | 5 | 5190-6863 | 5190-6864 | 5190-6865 | 5190-6866 | 5190-6867 | 5190-6868 |
| 21.2 x 50, Guard | 5 | 5190-6869 | 5190-6870 | 5190-6871 | 5190-6872 | 5190-6873 | 5190-6874 |
| 7.8 x 300 | 5 | 5190-2516 | 5190-2521 | 5190-2526 | 5190-2531 | 5190-2536 | 5190-2541 |
| 7.8 x 150 | 5 | 5190-2517 | 5190-2522 | 5190-2527 | 5190-2532 | 5190-2537 | 5190-2542 |
| 7.8 x 50, Guard | 5 | 5190-2520 | 5190-2525 | 5190-2530 | 5190-2535 | 5190-2540 | 5190-2545 |
| 4.6 x 300 | 5 | 5190-2518 | 5190-2523 | 5190-2528 | 5190-2533 | 5190-2538 | 5190-2543 |
| 4.6 x 150 | 5 | 5190-2519 | 5190-2524 | 5190-2529 | 5190-2534 | 5190-2539 | 5190-2544 |
| 4.6 x 50, Guard | 5 | 5190-6857 | 5190-6858 | 5190-6859 | 5190-6860 | 5190-6861 | 5190-6862 |



Bio-Monolith Protein A Column, 5069-3639

TITER DETERMINATION

Affinity chromatography is a powerful technique that takes advantage of highly specific molecular interactions, frequently between specific proteins (e.g. antigen/antibody). Agilent offers several specialty affinity products; a monolithic Protein A column for the isolation and quantitation of IgG and a series of Multiple Affinity Removal Systems for the elimination of high abundance proteins in biological samples.

Agilent Bio-Monolith Protein A HPLC Columns

- Designed for the analytical separation of all IgG (human and mouse), except for IgG class 3
- Separations independent of flow rate; no diffusion, no pores, and no void volume make transport between mobile and stationary phase very rapid
- Extremely fast separations speed up method development time and decrease costs
- Locking in method parameters takes significantly less time and buffer

Agilent Bio-Monolith Protein A HPLC columns are part of the Agilent Bio-Monolith column family. Protein A Bio-Monolith columns are compatible with Waters HPLC and preparative LC systems

TIPS & TOOLS

For more information on salt tolerance for mAb binding and acidic buffers compatibility for mAb elution on Agilent Bio-Monolith Protein A columns, see: *Agilent Bio-Monolith Protein A Monitors Monoclonal Antibody Titer from Cell Cultures* (publication 5991-2990EN)

www.agilent.com/chem/library

Column Specifications

| | |
|-----------------------------------|---|
| Dimensions | 5.2 mm x 4.95 mm |
| Column volume | 100 µL |
| Maximum pressure | 150 bar (15 MPa, 2,200 psi) |
| Temperature min/max | Operating: 2-40 °C Storage: 2-8 °C |
| Recommended pH | Operating range: 2-13 Cleaning-in-place: 1-14 |
| Materials of construction | Hardware: stainless steel Packing: poly(glycidyl methacrylate-co-ethylene dimethacrylate) highly porous monolith |
| Color ring identifier | Bio-Monolith Protein A: white |
| Shelf life/expiration date | Protein A: 12 months |

Bio-Monolith Protein A

| Column | Description | Key Applications | Part No. |
|------------------------|---|--|-----------------|
| Bio-Monolith Protein A | The Protein A affinity column is designed for the analytical separation of IgG1 and IgG2 (human and mouse). | Quantitative determination of IgG (fermentation titer calculation) | 5069-3639 |

TIPS & TOOLS

Further information can be found in:

mAb Titer Analysis with the Agilent Bio-Monolith Protein A Column (publication 5991-5135EN)

Agilent Bio-Monolith Protein A Monitors Monoclonal Antibody Titer from Cell Cultures (publication 5991-2990EN)

Cell Clone Selection Using the Agilent Bio-Monolith Protein A Column and LC/MS (publication 5991-5124EN)

Cell Culture Optimization Using an Agilent Bio-Monolith Protein A Column and LC/MS (publication 5991-5125EN)

www.agilent.com/chem/library



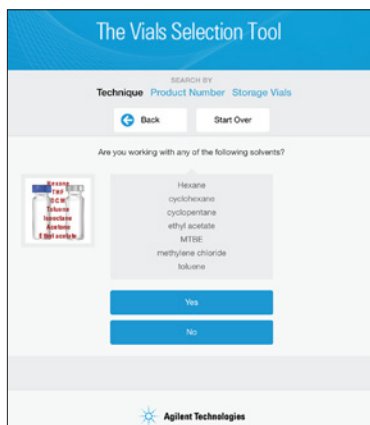
VIALS AND CLOSURES

Agilent vials and closures are thoroughly tested to ensure the highest level of quality. Additionally, Agilent vials are designed for use in a wide range of Waters LCs.

- Manufactured in an ISO 9001 certified facility
- Made from First Hydrolytic Type 1 Class A or Class B borosilicate glass, which conforms to US FDA, USP, and EU Pharmacopeia standards
- Protected by proprietary packaging with a crush barrier to reduce vial breakage
- Packaged in material that has been tested and selected for cleanliness
- Subjected to rigorous end-of-line sampling and quality control procedures to ensure all vials remain within specifications
- Compatible with a wide variety of autosamplers regardless of make and model

TIPS & TOOLS

Let the experts in chromatography help you select the right vials and closures for your application. Select your vial at www.selectvials.chem.agilent.com



Vials for Waters

| Similar to Waters Part No. | Part No. |
|----------------------------|-----------|
| 186001421 | 8010-0565 |
| 186001422 | 8010-0564 |
| 186000307C | 8010-0542 |
| 186000838C | 8010-0553 |
| 186000847C | 8010-0543 |
| 1860 01133C | 8010-0554 |

The table below illustrates that the Agilent vial portfolio is compatible with a wide range of Waters autosamplers.

Vial Compatibility Chart

| Autosampler | 8 mm Screw Top | 9 mm Screw Top | 15 x 45 mm, 4 mL | 11 mm Crimp Top |
|---------------|----------------|----------------|------------------|-----------------|
| 717 Plus | | | | ✓ |
| ACQUITY | ✓ | ✓ | | ✓ |
| Alliance 2690 | ✓ | ✓ | | |
| CapLC | ✓ | ✓ | | ✓ |
| WISP | | | ✓ | |

Vial Septum Chemical Compatibility Chart

| | PTFE | PTFE/Silicone | PTFE/ Silicone/PTFE* | PTFE/Red Rubber | Viton | PTFE/Butyl |
|---|------|---------------|----------------------|-----------------|-------|------------|
| Acetonitrile | ✓ | ✓ | ✓ | ✓ | | ✓ |
| Hydrocarbons (hexane, heptane, methane) | ✓ | | ✓ | ✓ | ✓ | |
| Methanol | ✓ | ✓ | ✓ | ✓ | | ✓ |
| Benzene | ✓ | | ✓ | | ✓ | |
| THF | ✓ | | ✓ | | | |
| Toluene | ✓ | | ✓ | | | |
| DMF | ✓ | ✓ | ✓ | | | ✓ |
| DMSO | ✓ | ✓ | ✓ | | | ✓ |
| Ether | ✓ | ✓ | ✓ | | | |
| Chlorinated solvents (methylene chloride) | ✓ | | ✓ | | ✓ | |
| Alcohols (ethanol) | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Acetic acid | ✓ | ✓ | ✓ | | | ✓ |
| Acetone | ✓ | ✓ | ✓ | | | |
| Phenol | ✓ | ✓ | ✓ | | ✓ | ✓ |
| Cyclohexane | ✓ | | ✓ | ✓ | ✓ | |

*PTFE/silicone/PTFE has the same chemical compatibility of PTFE ONLY UNTIL PUNCTURED

Agilent provides this septa compatibility information as a guide and starting reference point. We realize that chemical compatibility can vary depending on the concentration of the solvent, temperature, molecular weight of the solvent, and other factors. Therefore, we recommend that you try a variety of septa to determine the best one for your analysis.

Vial Cap and Septum Compatibility Chart

| | Thin PTFE | PTFE/ Silicone* | PTFE/ Silicone/PTFE* | PTFE/ Red Rubber | Viton | Butyl | High Performance Septa |
|-----------------------------|---|---|--|---|---|--|---|
| Temperature Range | Up to 260 °C | -40 °C to 200 °C | -40 °C to 200 °C | -40 °C to 90 °C | -40 °C to 260 °C | -50 °C to 150 °C | Up to 300 °C for up to 1 hour |
| Use for multiple injections | No | Yes | Yes | No | No | No | Yes |
| Price | Very economical | Economical | Most expensive | Very economical | Economical | Economical | More expensive |
| Resistance to coring | None | Excellent | Excellent | None | None | None | Excellent |
| Recommended for storage | No | Yes | Yes | No | No | No | No |
| Best for | Superior chemical inertness, short cycle times, and single injections | Most common HPLC and GC analyses, not as resistant to coring as P/S/P | Superior performance for ultra trace analysis, repeat injections, internal standards | Chlorosilanes, more economical option for single injections | Chlorinated solvents, higher temperatures | Organic solvents, acetic acids; impermeable to gases | High temperature headspace applications |

*Agilent silicone is platinum cured (versus peroxide cured), making it more inert and less likely to interact with samples

To obtain an optimal fit and seal of any vial and closure, we strongly recommend buying an entire vial assembly (including a vial, cap, and septum, as well as a vial insert if needed). Please avoid mixing and matching any vials with any other brand of closures, or any closures with any other brand of vials.

AUTOSAMPLER SYRINGES

Autosampler Syringes

| Model | Volume (µL) | Description | Needle Gauge/ Length (mm)/ Tip | Similar to Waters Part No. | Syringe Part No. | Replacement Needle Part No. | Similar to Waters Part No. | Replacement Plunger Part No. |
|--|----------------------------|----------------------------|--------------------------------------|----------------------------------|---------------------|-----------------------------------|----------------------------------|------------------------------------|
| 2777 Compact Sample Manager 2777 Sample Manager | 10 | Fixed needle | 22s/51/3 | 430000859 | 8010-0445* | | 700002212 | 8010-0457, 10/pk |
| | 25 | Fixed needle, gas tight | 22s/51/3 | 430000861 | 8010-0441 | | 700002213 | 8010-0458, 10/pk |
| | 100 | Fixed needle, gas tight | 22s/51/3 | 430000864 | 8010-0442* | | 700002214 | 8010-0459, 10/pk |
| | | Fixed needle, gas tight | 22s/51/3 | 430000863 | 8010-0446* | | 700002214 | 8010-0459, 10/pk |
| | 250 | Fixed needle, gas tight | 22s/51/3 | 430000865 | 8010-0467 | | 700002215 | 8010-0456, 10/pk |
| | 500 | Fixed needle, gas tight | 22s/51/3 | 430000866 | 8010-0468 | | 700002216 | 8010-0460, 10/pk |
| | 1,000 | Fixed needle, gas tight | 22s/51/3 | 430000867 | 8010-0443 | | 700002217 | 8010-0455, 10/pk |
| 2,500 | Fixed needle, gas tight | 22s/51/3 | 430000868 | 8010-0444 | | 700002218 | 8010-0448, 1/pk | |
| 510 HPLC Pump 515 HPLC Pump 600 MultiSolvent Delivery System CapLC System CapLC XE System | 10,000 | Luer Lock | No needle | WAT025559 | 8005-0414 | | | |

*Barrel od is 6.7 mm. All other 10, 25, and, 100 µL syringes have 7.9 mm od.

(Continued)

The cross references to the Waters part numbers listed here serve as a recommendation that the Agilent products are viable alternatives to Waters products. Agilent products are compatible with the corresponding Waters instruments, although in some cases, the Agilent products may have slightly different designs as compared to the Waters counterparts. All Agilent supplies are backed by the Agilent 90-day money-back warranty.



Autosampler syringe, 100 µL fixed needle, 8010-0442

Autosampler Syringes, 1/pk

| Model | Volume (µL) | Description | Needle Gauge/ Length (mm)/ Tip | Similar to Waters Part No. | Syringe Part No. | Replacement Needle Part No. | Similar to Waters Part No. | Replacement Plunger Part No. |
|--|--------------------|---|---|---|-----------------------------|--|---|---|
| 600 MultiSolvent Delivery System Rheodyne Injector | 25 | Removable needle, gas tight | 22s/51/3 | WAT033381 | 8005-0416 | | | 8005-0422 |
| 2690 Separations Module | 25 | Chem (1/4-28 UNF screw threads), gas tight | No needle | WAT077343 | 8005-0420 | | | |
| 2690D Dissolution Separations Module | | | | | | | | |
| 2695 Separations Module | | | | | | | | |
| 2695D Dissolution Separations Module | | | | | | | | |
| 2790 Separations Module | 250 | Chem (1/4-28 UNF screw threads), gas tight | No needle | WAT073109 | 8005-0419 | | | |
| 2795 Separations Module | | | | | | | | |
| LC Module 1 717/717plus Autosampler | | | | | | | | |
| Rheodyne Injector | 100 | Removable needle, gas tight | 22s/51/3 | WAT033383 | 8005-0417 | 8005-0418, 6/pk | | 8005-0423 |

The cross references to the Waters part numbers listed here serve as a recommendation that the Agilent products are viable alternatives to Waters products. Agilent products are compatible with the corresponding Waters instruments, although in some cases, the Agilent products may have slightly different designs as compared to the Waters counterparts. All Agilent supplies are backed by the Agilent 90-day money-back warranty.



SAMPLE PREPARATION

Reliably extract and concentrate samples from complex matrices

Sample preparation is an essential part of successful chromatography. It extends column lifetime, reduces the need for repeated samples, and minimizes interferences that can jeopardize your separation, detection, and quantification.

Agilent offers the most complete line of sample prep products across the full spectrum of instrumentation. These include:

- **Bond Elut SPE products**—selectively remove interferences and/or analytes from challenging matrices. They feature trifunctional bonding chemistry for greater stability—plus a three-tier QC process that confirms the correct particle size. Choose from the largest selection of sorbent formats in the market today.
- **Pre-packaged QuEChERS kits**—make sample preparation faster, easier, and more reliable. Options include extraction kits with pre-weighted salts in anhydrous packets, dispersive kits that accommodate aliquot volumes specified by AOAC/EN methods, and ceramic homogenizers that promote consistent extraction and recovery.
- **Enhanced Matrix Removal**—Lipid is a unique sorbent that selectively removes lipids in complex matrices and challenging high-fat samples. You can effectively remove lipids interfaces without losing your analytes, improving data quality and confidence in your results.
- **Filtration products** improve both system performance and analytical quality and prevent extractables or other contaminants from damaging the integrity of your samples. Choose from the industry's widest variety of membrane types and pore sizes to suit your applications.



Typical Matrices

Plasma, urine, biological fluids,
and aqueous samples

Primary Extraction Mechanism

Non-polar

Bond Elut Plexa Polymeric SPE

Bond Elut Plexa is a family of polymeric SPE products, designed for simplicity, improved analytical performance and ease-of-use. Its uniqueness lies in the novel hydroxylated exterior, hydrophobic interior and advanced polymeric architecture.

Bond Elut Plexa

Bond Elut Plexa is a nonpolar divinylbenzene-based neutral polymeric sorbent. This sorbent is the best choice for nonionic extraction of a wide range of acidic, neutral, and basic analytes from different matrices.

Bond Elut Plexa PCX

Bond Elut Plexa PCX is a cation exchanger with mixed-mode sorbent characteristics and is therefore suitable for the extraction and cleanup of weak bases from biofluids.

Bond Elut Plexa PCX demonstrates the same excellent particle size distribution and integrity as Bond Elut Plexa. A highly controlled sulfonation process results in zero fines for Bond Elut Plexa PCX.

Bond Elut Plexa PAX

Bond Elut Plexa PAX is an anion exchanger based on the same innovative base polymer particle technology as the other members of the Plexa SPE family. This advanced material offers excellent flow characteristics due to its monodisperse particle size distribution, affording superior ease-of-use, with minimal clogging of the packed bed. The amide-free particle technology does not provide binding sites for endogenous interferences such as proteins and lipids.

TIPS & TOOLS

Tabless (flangeless) cartridges are suitable for use with many automated SPE systems. Tabless products are typically designated with a "T" in the part number. If you need a tabless cartridge and do not see a part number listed, please contact your nearest Agilent office or Agilent Representative to discuss custom options.

| Bond Elut Plexa | | |
|--|-------------|-----------------|
| Description | Unit | Part No. |
| Straight Barrel Cartridges | | |
| 30 mg, 1 mL | 100/pk | 12109301 |
| 30 mg, 1 mL, tabless | 100/pk | 12109301T |
| 30 mg, 3 mL | 50/pk | 12109303 |
| 60 mg, 1 mL | 100/pk | 12109601 |
| 60 mg, 3 mL | 50/pk | 12109603 |
| 200 mg, 3 mL | 50/pk | 12109610 |
| 200 mg, 6 mL | 30/pk | 12109206 |
| 500 mg, 3 mL | 30/pk | 12109703 |
| 500 mg, 6 mL | 30/pk | 12259506 |
| Bond Elut Jr | | |
| 200 mg | 50/pk | 12169610B |
| Mega Bond Elut Plexa | | |
| 500 mg, 12 mL | 20/pk | 327832 |
| Other Formats | | |
| Bond Elut Plexa Prospekt cartridge, 2 mm | 96/pk | 12221305 |
| Bond Elut Plexa 800 Series cartridge | 96/pk | 12281305 |
| Gerstel, 60 mg, 3 mL | 50/pk | 167816G |
| Gerstel, 200 mg, 3 mL | 50/pk | 167822G |

| Bond Elut Plexa 96-well Plates | | |
|---------------------------------------|--------------|--------------|
| Description | 10 mg | 30 mg |
| Round-well, 1 mL | A4969010 | A4969030 |
| Square-well, 2 mL | A3969010 | A3969030 |



Typical Matrices

Plasma, urine, biological fluids,
and aqueous samples

Primary Extraction Mechanism

Mixed mode: non-polar and cation exchange

Bond Elut Plexa PCX

- Faster flow rates improve productivity
- Extraction cleanliness and reduced interference improve precision
- Simplified, single method for ease-of-use

Bond Elut Plexa PCX is another milestone in the development of simple and robust SPE methods. Plexa PCX uses a polymeric cation-exchange resin that combines the outstanding properties of Bond Elut Plexa—superior flow characteristics and improved analytical performance—with strong cation-exchange functionalities. This mixed-mode SPE sorbent removes neutral and acidic interferences from the matrix, concentrates basic analytes and therefore improves sensitivity in the determination of basic compounds.

The Plexa PCX particles are near monodispersed, resulting in homogenous packing. Reproducible results are the norm, with very good tube-to-tube and well-to-well performance. Ion suppression is reduced because the highly polar, hydroxylated polymer surface is entirely amide-free and does not provide binding sites for endogenous species such as proteins and lipids.

Plexa PCX comes with a simple, single method for basic drugs that offers improved recoveries, cleaner extracts, and reduced method development time and cost. Flow rate is improved because Plexa PCX particles have much narrower particle size distribution with no fines to cause blockages.

Typical Method for Bond Elut Plexa PCX

Sample:

100 µL plasma

Pretreatment:

Dilute 1:3 with 2% H₃PO₄

Conditioning:

1. 500 µL MeOH
2. 500 µL H₂O

Washes:

Acidic wash: 500 µL aqueous
2% formic acid

Neutral wash: 500 µL CH₃OH/CH₃CN
(1:1, v/v)

Elution:

500 µL CH₃OH/CH₃CN + 5% NH₃
(28-30%)

Volumes stated are for Bond Elut, 96-well, 30 mg,
1 mL, p/n A4968030.

Bond Elut Plexa PCX

| Description | Unit | Part No. |
|--|--------|-----------|
| Straight Barrel Cartridges | | |
| 30 mg, 1 mL | 100/pk | 12108301 |
| 60 mg, 1 mL | 100/pk | 12108601 |
| 30 mg, 3 mL | 50/pk | 12108303 |
| 60 mg, 3 mL | 50/pk | 12108603 |
| 60 mg, 3 mL, tabless | 50/pk | 12108603T |
| 200 mg, 6 mL | 30/pk | 12108206 |
| 500 mg, 6 mL | 30/pk | 12258506 |
| Other Formats | | |
| Bond Elut Plexa PCX Prospekt cartridge, 2 mm | 96/pk | 12221306 |
| Bond Elut Plexa PCX 800 Series cartridge, 2 mm | 96/pk | 12281306 |
| Gerstel | 50/pk | 168016G |

Bond Elut Plexa 96-well Plates

| Description | 10 mg | 30 mg |
|-------------------|----------|----------|
| Round-well, 1 mL | A4968010 | A4968030 |
| Square-well, 2 mL | A3968010 | A3968030 |



TIPS & TOOLS

View the core concepts of SPE and demonstrations of sample preparation, please visit www.agilent.com/chem/spevideo

Typical Matrices

Plasma, urine, biological fluids,
and aqueous samples

Primary Extraction Mechanism

Mixed mode: non-polar and anion exchange

**Typical Method for
Bond Elut Plexa PAX****Sample:**

100 µL human plasma

Pretreatment:

Dilute 1:3 with 2% NH₄OH

Conditioning:

1. 500 µL MeOH
2. 500 µL H₂O

Washes:

1. 500 µL H₂O
2. 500 µL MeOH

Elution:

500 µL 5% formic acid:MeOH

Volumes stated are for Bond Elut, 96-well, 1 mL,
p/n A4967010.

Bond Elut Plexa PAX

- Mixed mode, non-polar polymeric anion exchanger offers high level of analyte selectivity
- Exclusion of endogenous interferences offers superior cleanliness and minimizes ion suppression
- Simple, single method for ease-of-use, reduces method development time

Bond Elut Plexa PAX is a polymeric anion-exchange product (PAX) that sets the performance standard in analyte cleanup and reproducibility for polar and non-polar acidic analytes. Existing polymeric anion-exchange sorbents can exhibit a broad range of ion-exchange capacity from batch to batch, leading to method irreproducibility and compromised data. Plexa PAX particles are functionalized using a proprietary process, which allows anion exchange loadings to be controlled with a very high degree of reproducibility, giving more robust performance across the lifetime of your compound study or method.

This Plexa PAX polymeric mixed-mode SPE product comes with a simple, single method for non-polar acidic and polar acidic analytes that offers excellent clean up, even in complex matrices such as plasma. The optimized anion exchange method offers clean extracts, high recoveries, and low RSDs, reducing method development time, sample repeats, and overall cost per sample in the process.

Bond Elut Plexa PCX

| Description | Unit | Part No. |
|-----------------------------------|--------|----------|
| Straight Barrel Cartridges | | |
| 30 mg, 1 mL | 100/pk | 12107301 |
| 60 mg, 1 mL | 100/pk | 12107601 |
| 30 mg, 3 mL | 50/pk | 12107303 |
| 60 mg, 3 mL | 50/pk | 12107603 |
| 200 mg, 6 mL | 30/pk | 12107206 |
| 500 mg, 6 mL | 30/pk | 12257506 |

Bond Elut Plexa 96-well Plates

| Description | 10 mg | 30 mg |
|-------------------|----------|----------|
| Round-well, 1 mL | A4967010 | A4967030 |
| Square-well, 2 mL | A3967010 | A3967030 |



Bond Elut QuEChERS

Agilent Bond Elut QuEChERS Kits make sample prep as easy as 1- 2- 3. Prepackaged QuEChERS kits are an easy way to capture the time saving benefits of QuEChERS sample preparation.

- **Extraction kits** with pre-weighed anhydrous salts in sealed packets allow you to add salts after you add organic solvent to your sample—avoiding an exothermic reaction that can compromise analyte recovery.
- **Dispersive kits** with sorbents and salts supplied in 2 or 15 mL centrifuge tubes accommodate the aliquot volumes specified by current AOAC and EN methodologies.
- **Universal dispersive kits** provide excellent recoveries and reproducibility for all types of fruits and vegetables.
- **EMR—Lipid** selectively removes lipids and high fat samples and challenging complex matrices, providing cleaner samples and greater confidence in results.
- **Ceramic homogenizers** break up salt agglomerates, promoting consistent sample extraction and increasing product recovery during extraction and dispersion; shaking time reduced from 60 to 20 seconds.

To support the wide range of QuEChERS product options, we offer many applications featuring the QuEChERS approach. *The Agilent Bond Elut QuEChERS Food Safety Applications Notebook: Volume 2* (publication 5990-4977EN) includes many of these applications. You will find the applications grouped according to the standard method associated with the application, such as AOAC, EN, or the original method, along with a section for other approaches. A handy index also lets you search this guide based on matrix type and analyte class.

TIPS & TOOLS

Find the best QuEChERS products for your applications

Identify your ideal option by making selections based on current QuEChERS sample preparation product, matrix, or official QuEChERS method, at www.agilent.com/chem/SelectQuEChERS



QuEChERS AOAC 2007.01 extraction kit, 5982-5755



Ceramic homogenizer for 50 mL tubes, 5982-9313

QuEChERS Extraction Kits

Step 1: Extraction

Choose the extraction salt packet based on your method of analysis, AOAC or EN. The buffered extraction salts are amenable for more labile pesticides. Adding solvent and then salts to a comminuted fruit or vegetable sample (10 or 15 g) enables you to extract the pesticides of interest into the organic layer. Agilent pre-packages its QuEChERS salts and buffers in anhydrous packages. This allows you to add them after adding your solvent to the sample, as specified in QuEChERS methods.

In the tables below, the “CH” products contain the appropriately sized ceramic homogenizer for those particular kits.

QuEChERS Extraction Kits

| Method | Buffered | Contents | Ceramic Homogenizers | With 50 mL Tubes 50/pk | Packets Only | |
|----------------------------|----------|--|----------------------|---------------------------|--------------|-----------|
| | | | | | 50/pk | 200/pk |
| AOAC 2007.01 | Yes | 6 g MgSO ₄ ; 1.5 g NaAcetate | Yes | 5982-5755CH | | |
| | | | No | 5982-5755 | 5982-6755 | 5982-7755 |
| Original (10 g samples) | No | 4 g MgSO ₄ ; 1 g NaCl | Yes | 5982-5550CH | | |
| | | | No | 5982-5550 | 5982-6550 | 5982-7550 |
| Original (15 g samples) | No | 6 g MgSO ₄ ; 1.5 g NaCl | Yes | 5982-5555CH | | |
| | | | No | 5982-5555 | 5982-6555 | 5982-7555 |
| EN 15662 | Yes | 4 g MgSO ₄ ; 1 g NaCl; 1 g NaCitrate; 0.5 g disodium citrate sesquihydrate | Yes | 5982-5650CH | | |
| | | | No | 5982-5650 | 5982-6650 | 5982-7650 |
| Acrylamides* | No | 4 g MgSO ₄ ; 0.5 g NaCl | No | 5982-5850 | | |
| Veterinary Drugs** | No | 4g Na ₂ SO ₄ ; 1 g NaCl | No | 5982-0032 | | |

*Katerina Mastovaka and Steven J. Lehotay have done work to extend the scope of QuEChERS beyond fruits and vegetables⁽¹⁾, using it to extract acrylamides in potato chips and other fried foods.

**See: *Screening 36 Veterinary Drugs in Animal Origin Food by LC/MS/MS Combined with Modified QuEChERS Method* (publication 5991-0013EN).

1: Rapid Sample Preparation Method for LC-MS/MS or GC-MS Analysis of Acrylamides in Various Food Matrices. *J. Agric. Food Chem.*, 2006, 54, 7001-7008.



QuEChERS dispersive kit, 5982-5022



QuEChERS dispersive kit, 5982-5022CH



QuEChERS Dispersive Kits, Fruits and Vegetables

Step 2: Dispersive SPE Cleanup

Select the Dispersive SPE kit suited to the type of food being analyzed and the method you are following. In this step, an aliquot of the sample extract from Step One is added to a 2 or 15 mL centrifuge tube containing a small amount of SPE sorbent and MgSO₄. The sorbent will extract interfering matrix materials from the sample, while the MgSO₄ helps remove excess water and improves analyte partitioning. Select kits are now available with ceramic homogenizers (2 per tube).

QuEChERS Dispersive Kits, Fruits and Vegetables

| Kit | Size | Unit | AOAC 2007.01 Method | European Method EN 15662 |
|---|-------|--------|--|---|
| | | | Kit Contents Part No. | Kit Contents Part No. |
| General fruits and vegetables: Removes polar organic acids, some sugars, and lipids | 2 mL | 100/pk | 50 mg PSA 150 mg MgSO ₄ 5982-5022 | 25 mg PSA 150 mg MgSO ₄ 5982-5021 |
| | | | 5982-5022CH | 5982-5021CH |
| | 15 mL | 50/pk | 400 mg PSA 1200 mg MgSO ₄ 5982-5058 | 150 mg PSA 900 mg MgSO ₄ 5982-5056 |
| | | | 5982-5058CH | 5982-5056CH |
| Fruits and vegetables with fats and waxes: Removes polar organic acids, some sugars, more lipids, and sterols | 2 mL | 100/pk | 50 mg PSA 50 mg C18EC 150 mg MgSO ₄ 5982-5122 | 25 mg PSA 25 mg C18EC 150 mg MgSO ₄ 5982-5121 |
| | | | 5982-5122CH | 5982-5121CH |
| | 15 mL | 50/pk | 400 mg PSA 400 mg C18EC 1200 mg MgSO ₄ 5982-5158 | 150 mg PSA 150 mg C18EC 900 mg MgSO ₄ 5982-5156 |
| | | | 5982-5158CH | 5982-5156CH |

Part numbers ending in CH indicate tubes containing ceramic homogenizers.

(Continued)

QuEChERS Dispersive Kits, Fruits and Vegetables



| Kit | Size | Unit | AOAC 2007.01 Method | European Method EN 15662 |
|---|-------|--------|---|---|
| | | | Kit Contents Part No. | Kit Contents Part No. |
| Pigmented fruits and vegetables: Removes polar organic acids, some sugars and lipids, and carotenoids and chlorophyll; not for use with planar pesticides | 2 mL | 100/pk | 50 mg PSA 50 mg GCB 150 mg MgSO ₄ 5982-5222 5982-5222CH | 25 mg PSA 2.5 mg GCB 150 mg MgSO ₄ 5982-5221 5982-5221CH |
| | 15 mL | 50/pk | 400 mg PSA 400 mg GCB 1200 mg MgSO ₄ 5982-5258 5982-5258CH | 150 mg PSA 15 mg GCB 885 mg MgSO ₄ 5982-5256 5982-5256CH |



| | | | | |
|--|-------|--------|---|---|
| Highly pigmented fruits and vegetables: Removes polar organic acids, some sugars and lipids, plus high levels of carotenoids and chlorophyll; not for use with planar pesticides | 2 mL | 100/pk | 50 mg PSA 50 mg C18EC 150 mg MgSO ₄ 5982-5122 5982-5122CH | 25 mg PSA 7.5 mg GCB 150 mg MgSO ₄ 5982-5321 5982-5321CH |
| | 15 mL | 50/pk | 400 mg PSA 400 mg C18EC 1200 mg MgSO ₄ 5982-5158 5982-5158CH | 150 mg PSA 45 mg GCB 855 mg MgSO ₄ 5982-5356 5982-5356CH |



| | | | | |
|---|-------|--------|---|--|
| Fruits and vegetables with pigments and fats: Removes polar organic acids, some sugars and lipids, plus carotenoids and chlorophyll; not for use with planar pesticides | 2 mL | 100/pk | 50 mg PSA 50 mg GCB 150 mg MgSO ₄ 50 mg C18EC 5982-5421 5982-5421CH | |
| | 15 mL | 50/pk | 400 mg PSA 400 mg GCB 1200 mg MgSO ₄ 400 mg C18EC 5982-5456 5982-5456CH | |

Part numbers ending in CH indicate tubes containing ceramic homogenizers.

(Continued)

QuEChERS Dispersive Kits: Other Food Methods

| Kit | Size | Unit | AOAC 2007.01 Method | European Method EN 15662 |
|---|-------|--------|--|--------------------------|
| | | | Kit Contents Part No. | Kit Contents Part No. |
| Other Food Methods Removes biological matrix interferences, including hydrophobic substances (fats, lipids) and proteins | 2 mL | 100/pk | 25 mg C18 150 mg MgSO ₄ 5982-4921 5982-4921CH | |
| | 15 mL | 50/pk | 150 mg C18 900 mg MgSO ₄ 5982-4956 5982-4956CH | |
| All Food Types Removes all matrix interfering materials including polar organic acids, lipids, sugars, proteins, carotenoids, and chlorophyll | 2 mL | 100/pk | 50 mg PSA 50 mg C18 7.5 mg GCB 150 mg MgSO ₄ 5982-0028 5982-0028CH | |
| | 15 mL | 50/pk | 400mg PSA 400 mg C18 45 mg GCB 1200 MgSO ₄ 5982-0029 5982-0029CH | |
| Animal Origin Food Removes matrix interferences such as polar organic salts, sugars, lipids, and proteins | 15 mL | 50/pk | 50 mg PSA 150 mg C18EC 900 mg Na ₂ SO ₄ 5982-4950 | |



Part numbers ending in CH indicate tubes containing ceramic homogenizers.



TIPS & TOOLS

View the core concepts surrounding the QuEChERS method at www.agilent.com/chem/QuEChERSvideo



Bond Elut Enhanced Matrix Removal—Lipid

Interference from lipids is a problem for labs measuring trace residues in fatty foods or complex biological matrices. Lipids can build up in the instrument and column, decreasing lifetime and reducing analyte sensitivity due to ion suppression. The need for MS maintenance increases too, because of lipid deposits on the source.

The need for lipid removal is well understood, but current methods often sacrifice analyte recovery, removing some of your target analytes along with the lipids.

Now, you don't have to choose between lipid removal and analyte recovery, because innovative Agilent Enhanced Matrix Removal—Lipid delivers the most complete lipid removal AND analyte recovery of any sample prep product.

- **Higher quality results:** a cleaner sample profile leads to greater data integrity and confidence in results, faster data processing and fewer re-runs.
- **Improved productivity:** better sensitivity and signal-to-noise from fewer matrix interferences enables faster data processing and greater sample throughput.
- **Reduced lab costs:** cleaner samples using EMR—Lipid can offer up to 50% less MS source maintenance, giving you more time to analyze samples rather than spend time on costly troubleshooting, downtime and instrument repair.
- **Simplified workflows:** standardize on an easy-to-use single-sorbent procedure that maximizes analyte recovery from a wide variety of fatty samples.
- **Save time and money** by reducing material costs, inventory, training time, and documentation, to streamline lab efficiency.

The EMR—Lipid dispersive kit consists of 50 15mL centrifuge tubes containing 1 g of preweighed sorbent. This volume can easily accommodate the aliquots specified by AOAC and EN methods. The polishing kit contains 50 15mL centrifuge tubes containing 5g of preweighed polishing salts.

Learn more at www.agilent.com/chem/EMR-Lipid

Bond Elut EMR—Lipid

| Description | Part No. |
|--|-----------|
| Bond Elut dSPE Enhanced Matrix Removal—Lipid | 5982-1010 |
| Bond Elut Final Polish for Enhanced Matrix Removal—Lipid | 5982-0101 |



Captiva Filtration

Filtering samples before analysis can deliver longer column lifetime, less downtime, and optimal instrument performance, because even small amounts of particulate can clog your column inlet or contaminate your system. You can remove damaging particulates with Agilent Captiva premium syringe filters—a great choice for simple mechanical filtration.

Captiva premium syringe filters are designed to give you:

- **Greater productivity:** the unique design produces the industry’s fastest flow rates
- **High loading capacity:** handle more particulates and greater volumes than other manufacturers’ products
- **Lowest extractable levels:** virtually free of extractables under conditions specified by the certificate

All premium syringe filters are certified by LC. What’s more, the polyethersulfone and glass fiber filters are certified using LC/MS.

Premium Filters, 100/pk

| Description | Diameter (mm) | Pore Size (µm) | Certification | Housing | Part No. |
|-------------|---------------|----------------|---------------|---------------|-----------|
| PTFE | 4 | 0.2 | LC | Polypropylene | 5190-5082 |
| | 4 | 0.45 | LC | Polypropylene | 5190-5083 |
| | 15 | 0.2 | LC | Polypropylene | 5190-5084 |
| | 15 | 0.45 | LC | Polypropylene | 5190-5085 |
| | 25 | 0.2 | LC | Polypropylene | 5190-5086 |
| | 25 | 0.45 | LC | Polypropylene | 5190-5087 |
| Nylon | 15 | 0.2 | LC | Polypropylene | 5190-5088 |
| | 15 | 0.45 | LC | Polypropylene | 5190-5091 |
| | 25 | 0.2 | LC | Polypropylene | 5190-5092 |
| | 25 | 0.45 | LC | Polypropylene | 5190-5093 |
| PES | 4 | 0.2 | LC/MS | Polypropylene | 5190-5094 |
| | 4 | 0.45 | LC | Polypropylene | 5190-5095 |
| | 15 | 0.2 | LC/MS | Polypropylene | 5190-5096 |
| | 15 | 0.45 | LC | Polypropylene | 5190-5097 |
| | 25 | 0.2 | LC/MS | Polypropylene | 5190-5098 |
| | 25 | 0.45 | LC | Polypropylene | 5190-5099 |

(Continued)

Premium Filters, 100/pk

| Description | Diameter (mm) | Pore Size (µm) | Certification | Housing | Part No. |
|-----------------------|---------------|----------------|---------------|---------------|-----------|
| Regenerated Cellulose | 4 | 0.2 | LC | Polypropylene | 5190-5106 |
| | 4 | 0.45 | LC | Polypropylene | 5190-5107 |
| | 15 | 0.2 | LC | Polypropylene | 5190-5108 |
| | 15 | 0.45 | LC | Polypropylene | 5190-5109 |
| | 25 | 0.2 | LC | Polypropylene | 5190-5110 |
| | 25 | 0.45 | LC | Polypropylene | 5190-5111 |
| Cellulose Acetate | 28 | 0.2 | LC | MBS | 5190-5116 |
| | 28 | 0.45 | LC | MBS | 5190-5117 |
| Glass Microfiber | 15 | | LC/MS | Polypropylene | 5190-5120 |
| | 28 | | LC | MBS | 5190-5122 |



Econofilters, PES, 5190-5272

Econofilters, 1000/pk

| Description | Diameter (mm) | Pore Size (µm) | Housing | Part No. |
|------------------------|---------------|----------------|---------------|-----------|
| PVDF | 13 | 0.2 | Polypropylene | 5190-5261 |
| | 13 | 0.45 | Polypropylene | 5190-5262 |
| | 25 | 0.2 | Polypropylene | 5190-5263 |
| | 25 | 0.45 | Polypropylene | 5190-5264 |
| PTFE | 13 | 0.2 | Polypropylene | 5190-5265 |
| | 13 | 0.45 | Polypropylene | 5190-5266 |
| | 25 | 0.2 | Polypropylene | 5190-5267 |
| | 25 | 0.45 | Polypropylene | 5190-5268 |
| Nylon | 13 | 0.2 | Polypropylene | 5190-5269 |
| | 13 | 0.45 | Polypropylene | 5190-5270 |
| | 25 | 0.2 | Polypropylene | 5190-5271 |
| | 25 | 0.45 | Polypropylene | 5190-5272 |
| PES | 13 | 0.2 | Polypropylene | 5190-5273 |
| | 13 | 0.45 | Polypropylene | 5190-5274 |
| | 25 | 0.2 | Polypropylene | 5190-5275 |
| | 25 | 0.45 | Polypropylene | 5190-5276 |
| Polypropylene | 13 | 0.2 | Polypropylene | 5190-5277 |
| | 13 | 0.45 | Polypropylene | 5190-5278 |
| | 25 | 0.2 | Polypropylene | 5190-5279 |
| | 25 | 0.45 | Polypropylene | 5190-5280 |
| Regenerated Cellulose* | 15 | 0.2 | Polypropylene | 5190-5310 |
| | 15 | 0.45 | Polypropylene | 5190-5308 |
| | 25 | 0.2 | Polypropylene | 5190-5309 |
| | 25 | 0.45 | Polypropylene | 5190-5307 |

*Premium syringe filter in 1,000/pk



Captiva filter cartridges, glass fiber, A500401000

Captiva Filter Cartridges

| Pore Size (µm) | Filter Material | Volume (mL) | Unit | Part No. |
|----------------|---|-------------|--------|------------|
| 0.2 | Polyvinylidene fluoride and polypropylene | 3 | 100/pk | A5300002 |
| 0.45 | Polyvinylidene fluoride and polypropylene | 3 | 100/pk | A5307045 |
| | | 6 | 100/pk | A5060045 |
| 10 | Glass fiber | 10 | 100/pk | A500401000 |

Captiva Non-Drip Filter Cartridges

| Pore Size (µm) | Filter Material | Volume (mL) | Unit | Part No. |
|------------------------|-----------------|-------------|--------|----------|
| Non-Drip | | | | |
| 0.22 | Polypropylene | 3 | 100/pk | A5300063 |
| Non-Drip Lipids | | | | |
| 0.22 | Polypropylene | 3 | 100/pk | A5300635 |



Captiva 96-well filter plates, A5960045

TIPS & TOOLS

For more information on Agilent Captiva ND plates, please visit www.agilent.com/chem/captiva

TIPS & TOOLS

Using Captiva ND Lipids with methanol is an excellent replacement for acetonitrile as the precipitation solvent. Methods with methanol show better removal of lipids than with acetonitrile. Converting to methanol is advantageous when the supply or cost of acetonitrile is restrictive. Methanol can now be your solvent of choice for lipid removal.

For more information about solvents, see: *Agilent Captiva ND Lipids Sample Prep Choice of Precipitation Solvent: Acetonitrile versus Methanol* (publication 5991-0445EN).

Captiva 96-well Filter Plates

| Pore Size (µm) | Filter Material | Unit | Part No. |
|----------------|---|--------|-------------|
| 0.2 | Polypropylene | 5/pk | A5960002 |
| | Polypropylene | 100/pk | A5960002B |
| 0.45 | Polyvinylidene fluoride and polypropylene | 5/pk | A5967045 |
| | Polypropylene | 5/pk | A5960045 |
| | Polypropylene | 100/pk | A5960045B |
| 10 | Glass fiber | 5/pk | A596401000 |
| 20 | Polypropylene | 5/pk | A596002000 |
| | Polypropylene bulk pack | 100/pk | A596002000B |

Captiva ND 96-well Filter Plates

| Description | Unit | Part No. |
|--|------|----------|
| Captiva ND plate, 0.2 µm, polypropylene Recommended for methanol and acetonitrile | 5/pk | A5969002 |
| Captiva ND plate, 0.45 µm, polypropylene Suitable for acetonitrile only | 5/pk | A5969045 |

Captiva ND Lipids 96-well Filter Plates

| Description | Unit | Part No. |
|---|--------|------------|
| Captiva ND Lipids 96-well filtration plate | 100/pk | A59640002B |
| Captiva ND Lipids 96-well filter plate, 1 mL well | 1/pk | A59640002I |
| Captiva ND Lipids 96-well filter plate, 1 mL well | 5/pk | A59640002V |
| DuoSeal 96 96-well plate seals | 10/pk | A8961008 |



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| Consumables/Supplies Required for Repair,* including liners, seals, tubing, assemblies, and multipliers | ✓ | ✓ | ✓ | |
| Maintenance Services | | | | |
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| Agilent Remote Advisor-Report** | ✓ | ✓ | ✓ | |
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| Guaranteed Pass (OQ) | ✓ | ✓ | | |
| Discount when Bundling Re-qualification (RQ) | ✓ | ✓ | | |

*Per local parts replacement policy.

**Where available. Installation required. Installation fees waived when connecting minimum number of systems.

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