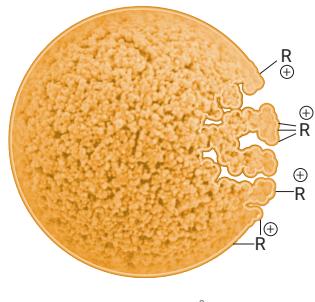


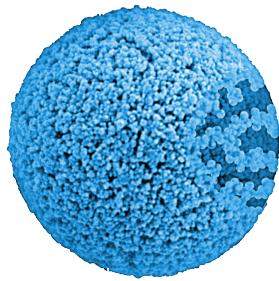
Oligonucleotide Purification Solutions

Agilent PLRP-S and PL-SAX HPLC columns





PL-SAX 1000 Å
Pore Size 1000 Å



PLRP-S 1000 Å
Pore Size 1000 Å

It's All About Scalability

Whether you're working on the bench or in manufacturing, Agilent PLRP-S and PL-SAX columns deliver robustness and reproducibility. What's more, they are available in pore sizes that are selective and appropriate for your unique oligonucleotide purification requirements.

A diverse class of molecules

Research has shown that synthetic oligonucleotides and nucleic acids are promising therapeutic agents as well as an essential part of next generation sequencing (NGS) for research and diagnostics.

These oligonucleotides can range in size, sequence complexity, and overall modifications. Small oligonucleotides can span a range of sizes from a few nucleotides to 40 or 50 bases, with varying DNA or RNA modifications. Beyond their primary structure, these molecules can be single or double stranded, and modified with molecular or biological tags. Larger oligonucleotides—including guide RNA or NGS primers and probes—can span from 50 to 200 bases, with varying modifications and tags for their particular use. RNA therapeutics can have sequences that are thousands of bases in length, synthesized through in-vitro production, requiring unique purification considerations.

Sequence-derived structure formations and impurities resemble the full-length product, so optimizing

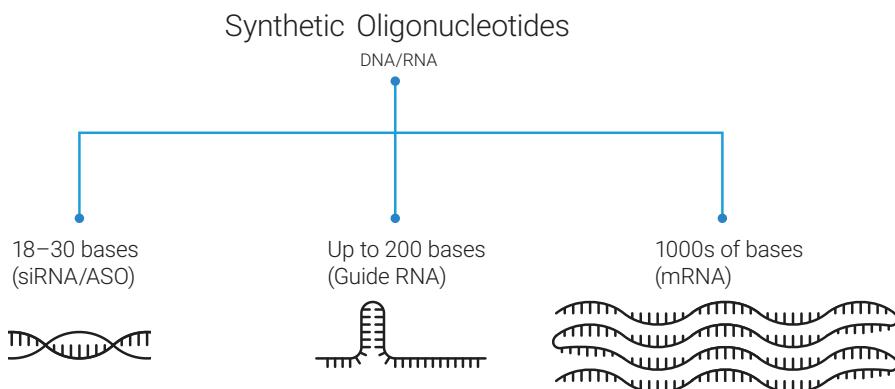
purification and analysis is essential when developing a final pure molecule. However, oligonucleotide diversity demands chemistries that can meet the challenges of purification and analysis through a range of particles and pore sizes.

Polymeric particles, such as PLRP-S and PL-SAX, are ideal for analyzing and purifying oligonucleotides. Because these materials are thermally and chemically stable, they enable diverse techniques:

- Working at elevated temperature enhances mass transfer, increasing resolution for longer oligonucleotides.
- Denaturing conditions, such as high pH, can be used to avoid aggregation of modified, self-complementary, and GC-rich oligonucleotides during analysis and purification.
- Performing anion exchange at high pH can suppress ionization of individual bases, enabling resolution of phosphodiester and phosphorothioate forms of the oligonucleotide.

Purification your way: siRNA to mRNA

Agilent recognizes the diversity of the oligonucleotide landscape, offering the optimal chemistry and pore size no matter your molecule size or purification scale.



Choosing the right pore size is an important factor for large molecules like oligonucleotides. Agilent offers a range of pore sizes to find the balance between resolution and binding capacity.

Pore sizes	siRNA/ASO	Guide RNA	mRNA
100 Å	●		
300 Å	●	●	
1000 Å	●	●	●
4000 Å		●	●

Agilent PLRP-S columns:

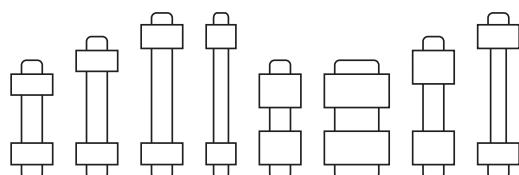
Ideal for your small-scale analysis, and scales as your production needs grow

- Ion pair reversed-phase chemistry
- Four pore sizes: 100 Å, 300 Å, 1000 Å, and 4000 Å
- Particles from 3 to 50 µm

Agilent PL-SAX columns:

The purity you need whether you are working with guide RNA or large nucleic acid molecules

- Best-in-class polymeric anion exchange media
- Two pore sizes: 1000 Å and 4000 Å
- Particles from 5 to 30 µm



Having the same quality stationary phase available in the scale needed is critical— from analytical dimensions, semi-prep and prep, to bulk material—Agilent PLRP-S and PL-SAX columns allow you to utilize the same optimized chemistry regardless of the scale of production.

Analytical characterization with PLRP-S columns



The Agilent 6545XT AdvanceBio LC/Q-TOF system:

Designed to handle multiple workflows

Whether you need to perform oligonucleotide analysis at the intact level, sequence and modification confirmation through fragment identification, or identify and quantify impurities, the 6545XT delivers.

It's the analytical cornerstone in a comprehensive set of tools designed for biopharma characterization.

Withstands high temperatures and pH

Agilent PLRP-S columns are available in a range of pore and particle sizes, all with identical chemistries and fundamental adsorptive characteristics. The particles are inherently hydrophobic, so there is no bonded phase required for reversed-phase separations. As such, PLRP-S particles can withstand extreme temperature and pH ranges that are incompatible with silica-based columns. That means you get the broadest allowable functionality for method development with particles as small as 3 µm.

PLRP-S has excellent binding capacity across five pore sizes: 100 Å, 300 Å, 1000 Å, and 4000 Å. This allows you to capture a wide range of oligonucleotides, whether you are looking at small antisense oligonucleotides or large mRNA.

In this example, we used an Agilent AdvanceBio 6545XT LC/Q-TOF to analyze poly-A tail sequences formed by e. coli poly-a polymerase (PAP). PAP is commonly used for post in-vitro transcription addition of polyA tails to mRNA, an essential component for translation.

Analysis of poly-A sequences extended by PAP on synthetic RNA Primer

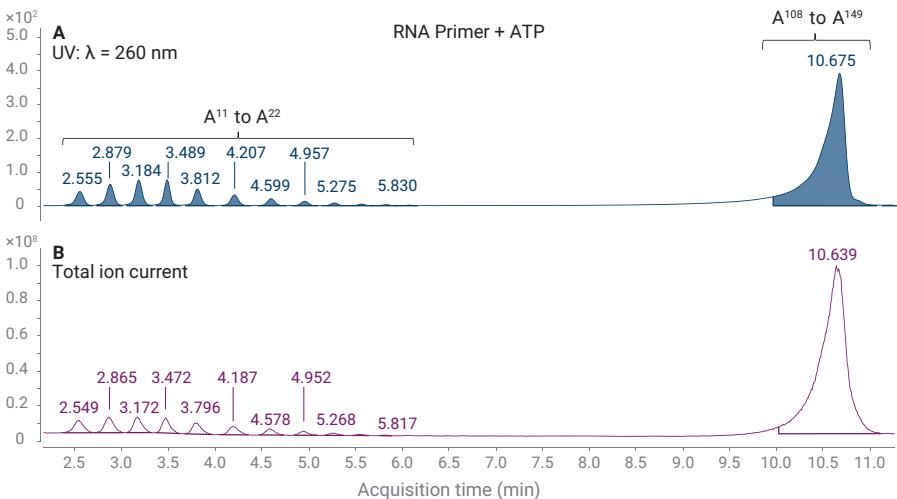


Figure 1. UV absorbance at 260 nm (A: reference = 360 nm) and total ion chromatogram (B) of RNA primers extended with PAP in the presence of ATP only. Separation was carried out on a PLRP-S column and shows bimodal distribution of two distinct populations.

Conditions

Instrument:	Agilent 1290 Infinity II LC system
Column:	Agilent PLRP-S, 5 µm, 2.1 × 50 mm, 1,000 Å
Solvent A:	15 mM dibutylamine + 25 mM HFIP in DI water
Solvent B:	15 mM dibutylamine + 25 mM HFIP in methanol
Gradient:	15% B 0–1 min, 45% B 1.1–10.5 min, 90% B 10.6–11.5 min
Flow rate:	0.4 mL/min
Temperature:	80 °C
Injection volume:	10–20 µL

Analytical characterization with PL-SAX columns

Delivering excellent resolution



Ion exchange analysis is often used for release testing of oligonucleotides, and offers robustness with common buffers and reagents found in QC labs.

Whether you are performing analytical characterization of oligonucleotide impurities or scaling up for future purification, PL-SAX delivers the flexibility you need. PL-SAX columns are composed of a strong anion exchange functionality covalently linked to a fully porous chemically stable polymer, extending its operating pH range and stability. In addition, the anion-exchange capacity is independent of pH, enabling separations that use denaturing conditions of temperature, organic solvent, and high pH for synthetic oligonucleotides.

The 5 µm media delivers high-resolution separations, while the 30 µm media is used for medium-pressure liquid chromatography. With pore sizes of 1000 and 4000 Å, PL-SAX offers high binding capacity and superior flow dynamics for oligonucleotides ranging from a few bases to thousands of bases in length.

Analytical separation of crude 25, 50, 75, and 100 mer

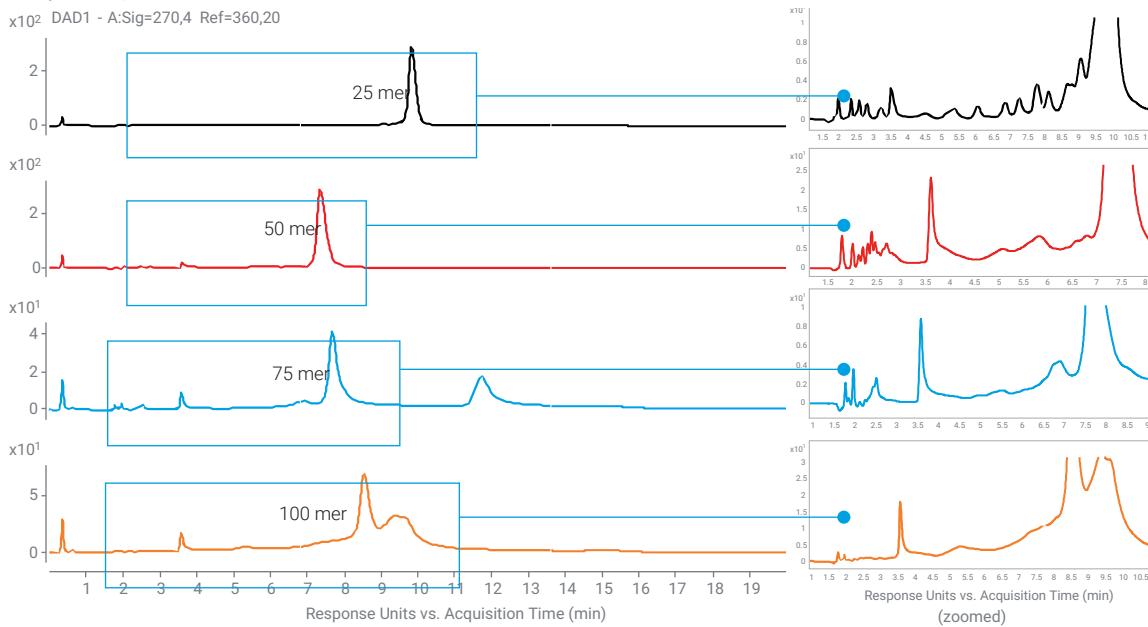


Figure 2. Analytical separation of crude 25, 50, 75, and 100 mer using PL-SAX 1000 Å, 5 µm, 2.1 x 50 mm column

Conditions

Solvent A:	10 mM Tris, pH 8.0 in water/ACN 10:90 (v:v)	Gradient:	20–40% B in 10 min, except for 25 mer which was conducted at 10–30% B
Solvent B:	2 M NaCl in solvent A	Sample:	1 mg/mL samples
Column temp:	30 °C unless otherwise noted	Injection:	2 µL except for 25 mer which was 1 µL
Flow rate:	0.5 mL/min		

PLRP-S: Providing the Scale and Pore Size for Your Purification Goals

PLRP-S polymeric materials are an ideal choice for ion pair reversed-phase oligonucleotide purification. They are physically and chemically robust and therefore well suited to the conditions required. Choosing the correct pore size for the length of oligonucleotide is important to optimize the binding capacity and overall oligonucleotide purification.

Available in a range of pore sizes (100 Å, 300 Å, 1000 Å, and 4000 Å), accessibility of very large molecules may be hindered in smaller pore sizes commonly seen across the market. Even though smaller pore size particles have higher internal surface area, if the molecule is too large to fit into the pores, the capacity will be compromised.

Purification of Single-Stranded RNA Oligonucleotides using the Agilent 1290 Infinity II Preparative LC system

By coupling PLRP-S and the Agilent 1290 Infinity II Preparative system, you can achieve ideal, efficient separations using ion pair reversed-phase methods. As you can see below in Figure 3, PLRP-S allows high sample loading, and the combined autosampler/fraction collector is ideal for large scale injections and flexible fraction collection.

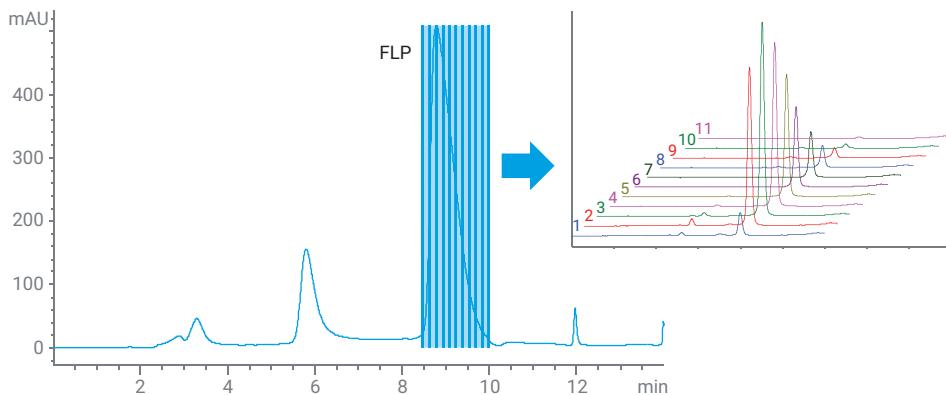


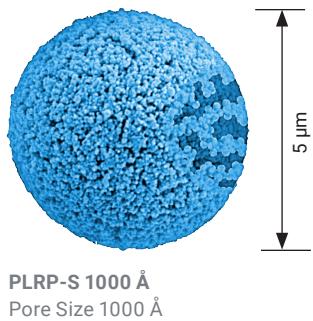
Figure 3. A chromatogram and overlay (UV 260nm) of an injection, fraction collection, and reanalysis of 11, 9 second fractions collected over the FLP.

Conditions for chromatographic parameters for purification

Mobile phase A: 0.1 M Hexylammonium acetate in water, pH = 7.0, + 10% urea
Mobile phase B: Acetonitrile
Flow rate: 30 mL/min
Gradient: Time (min) % B
0 32
0.26 32
10.10 42
11.09 100
12.07 100
13.06 32
Stop time: 14 min
Post time: 1.5 min
Injection volume: 4,000 µL
Temperature: Ambient
UV detection: 260 nm
No reference
5 Hz data rate
Fraction collection: Peak-based from 7.0 to 12.0 min, collecting 9 s time slices
UV threshold: 5 mAU
UV upslope: 2 mAU/s
UV downslope: 1 mAU/s

Conditions for chromatographic parameters for fraction reanalysis

Mobile phase A: 0.1 M Hexylammonium acetate in water, pH = 7.0
Mobile phase B: Acetonitrile
Flow rate: 1 mL/min
Gradient: Time (min) % B
0 28
8 36
9 100
10 100
11 28
Stop time: 11 min
Post time: 6 min
Injection volume: 10 µL
Temperature: 80 °C
UV detection: 260/4 nm
Reference 360/100 nm
5 Hz data rate



PLRP-S 1000 Å
Pore Size 1000 Å

Other particle sizes:
3 μm, 8 μm, 10 μm, 15 μm,
20 μm, 30 μm, 50 μm

Other pore sizes: 100 Å,
300 Å, 4000 Å

Choosing the right pore size

As the pore size of the particle increases, the corresponding surface area decreases. As such, the smaller 100 Å pore has the highest binding capacity for the smaller oligonucleotides (25, 50 mer), while larger pores provide increased binding capacity for larger oligonucleotides (75 mer) through increased permeability and flow dynamics. Therefore, it is important to balance pore size against target to maximize your purification (Figure 4).

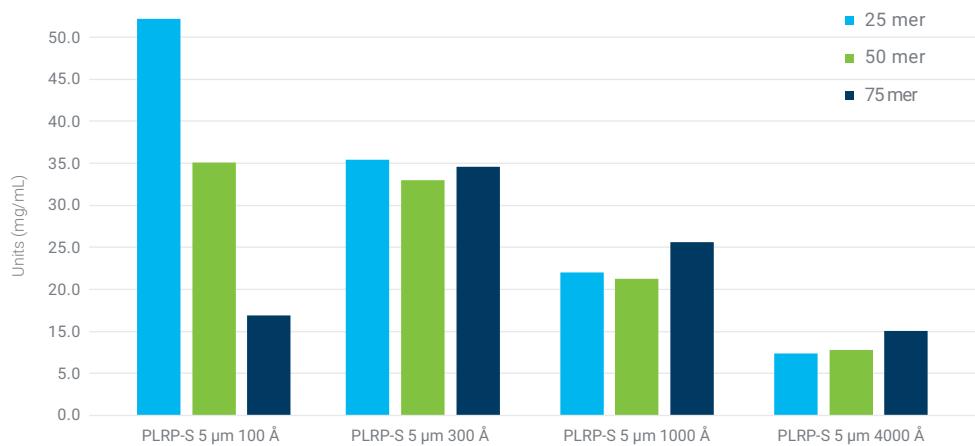


Figure 4. Illustrative binding capacity of 5 μm PLRP-S by pore size.



Maintain confidence in your results with Agilent oligonucleotide standards

Agilent standards are rigorously tested and manufactured using ISO certifications—including ISO 17025 and 17034. So you can calibrate with confidence and maximize accuracy.

Learn more at [www.agilent.com/
chem/oligonucleotide-standards](http://www.agilent.com/chem/oligonucleotide-standards)

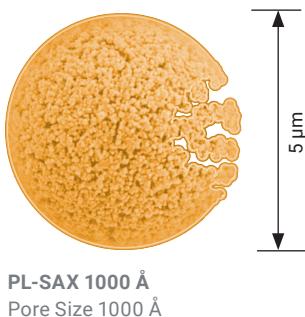
Dynamic binding capacity provides a useful indication of how a stationary phase may behave for oligonucleotide purification. It can help determine which material may give the best results for purifying a particular oligonucleotide, and give an indication to the column size that may be required.

Estimated loading capability by column internal diameter

Loading (mg) per 50 mm bed depth (based on 5% capacity)													
		PLRP-S 100 Å			PLRP-S 300 Å			PLRP-S 1000 Å			PLRP-S 4000 Å		
ID (mm)	Vol (mL)	25 mer	50 mer	75 mer	25 mer	50 mer	75 mer	25 mer	50 mer	75 mer	25 mer	50 mer	75 mer
2.1	0.17	0.4	0.3	0.1	0.3	0.2	0.3	0.1	0.1	0.2	0.1	0.1	0.1
4.6	0.83	2.0	1.3	0.5	1.3	1.2	1.2	0.7	0.7	0.9	0.3	0.3	0.4
7.5	2.2	5.2	3.3	1.3	3.4	3.1	3.3	1.9	1.8	2.3	0.8	0.9	1.1
25	24.5	58	37	15	37	34	36	21	20	25	9	10	12
50	98	232	148	58	149	137	145	83	80	101	36	38	50
100	393	927	591	234	597	550	581	334	318	404	145	153	198

Figure 5. Estimate of the amount of oligonucleotide (mg) that might be purified as an initial injection quantity from which optimization and validation is performed. Quantities were calculated using 5% of dynamic binding capacity determined from analytical runs.

Prep-to-process purification with PL-SAX columns



Other particle sizes:
8 μm, 10 μm, 30 μm

Other pore sizes: 4000 Å

Exceptional pH and thermal stability

Polymeric PL-SAX is chemically and thermally stable under diverse HPLC conditions. These robust ion-exchange columns can be used over a wide range of linear velocities, with fast loading of dilute solutions and wash cycles.

In addition, the increased binding capacity of PL-SAX makes it an attractive choice when scaling up and transitioning from small-scale ion pair reversed-phase purifications to more traditional buffering systems. Their high binding capacity helps you meet your yield requirements using aqueous buffer/non-volatile conditions.

You can also count on:

- Excellent reproducibility and long column life.
- Purifications over a wider pH range. Strong, hydrophilic ion exchange functionalities are covalently linked to a chemically stable polymeric particle.
- Rapid HPLC flow rates and equilibration for reduced purification cycle times.
- Faster sanitation and clean-up through increased column stability.
- High flexibility. The 1000 Å pore size is ideal for high-capacity purifications, while the 4000 Å gigaporous particles with improved mass transfer are best for large biomolecules such as mRNA.

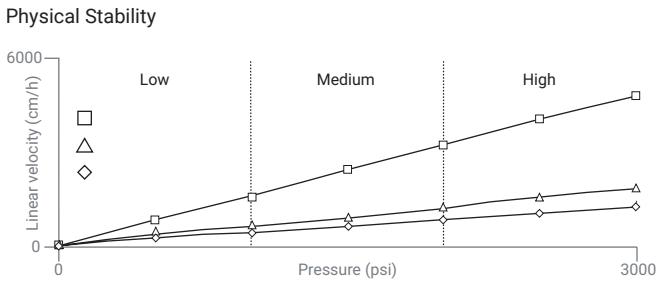


Figure 6. Pressure vs linear velocity. PL-SAX particles are physically robust and stable up to 6000 psi.

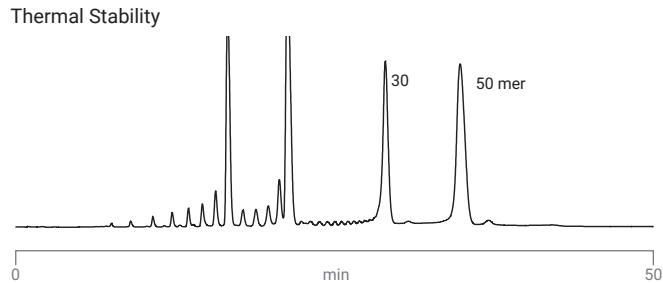


Figure 7. Separation of a poly T-standard at 60 °C. Thermal stability lets you use denaturing conditions and stabilizing/solubilizing agents to purify target synthetic oligonucleotides with self-complementary sequences.

Conditions

Column:	PL-SAX 1000 Å 8 μm, 4.6 x 50 mm
	Eluent A: 93% 0.1 M TEAA, pH 8.5/7% ACN
	Eluent B: 93% 0.1 M TEAA, 1 M ammonium chloride, pH 8.5/7% ACN
Gradient:	0–40% B in 10 min, 40–70% B in 14 min, 70–100% B in 25 min
Flow rate:	1.5 mL/min
Temperature:	60 °C



When scaling oligonucleotide purification, it is critical to select a particle and column dimension that will meet yield and throughput goals. Determination of dynamic binding capacity is a useful tool for determining the expected quantity of oligonucleotide that can be purified under a given set of conditions.

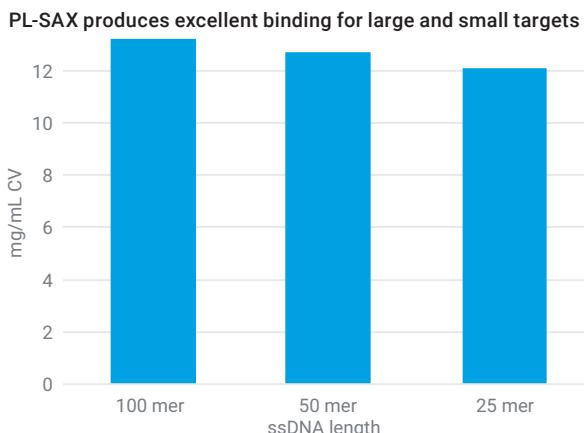


Figure 8. Illustrative dynamic binding capacity of PL-SAX 1000 Å, 8 µm for 100, 50, and 25 mer ssDNA. Binding capacity was measured at 25% of breakthrough curve by overloading the oligonucleotide of interest.

Representative loading quantity by column diameter for PL-SAX

Column id (mm) x 50 mm	25 mer DNA 5% Binding Capacity (nmoles)	50 mer DNA 5% Binding Capacity (nmoles)	100 mer DNA 5% Binding Capacity (nmoles)
2.1	14.55	6.97	3.37
4.6	71.03	34.01	16.45
7.5	189.1	90.6	43.8
25	2,100	1,006	486.5
50	8,401	4,023	1,946
100	33,607	16,091	7,785

Figure 9. Representative starting load quantities of 25, 50, and 100 mer oligonucleotide for AEX purification utilizing 5% of the dynamic binding capacity of PL-SAX. Loading quantities should be tested for each oligonucleotide and may allow for loading more or less depending on purification goals and impurity profile.

Following accurate DBC determination, injection of a low percent of the total DBC (typically 1–5%) can allow estimation of the total injection quantity per column volume that can be expected while scaling to prep columns. The percent of the DBC that can be used for purification injections is highly dependent on the impurity profile of the oligonucleotide, though 5% of the DBC is a good initial starting point by which optimization can be performed. No matter the quantity of oligonucleotide being purified, from µg to kg, PL-SAX is offered in a range of column dimensions and bulk media for your preparative needs.

Representative Dynamic Binding Capacity experiment using 100 mer DNA on PL-SAX

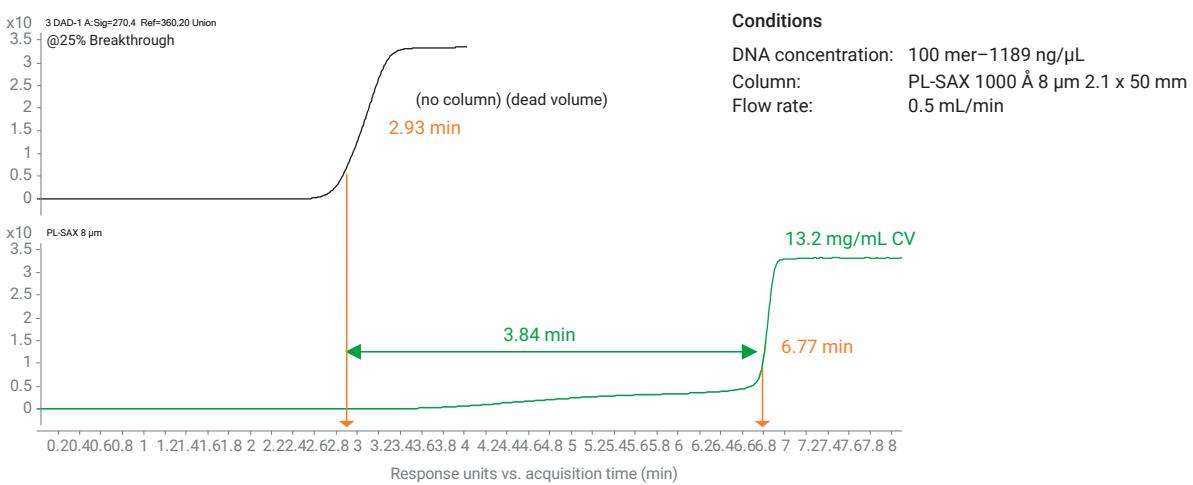


Figure 10. PL-SAX large pore size and high binding capacity ensures high purity and yield.

Separating guide RNA and modified oligonucleotides with PL-SAX Anion Exchange columns

With their 1000 Å and gigaporous totally porous particles, PL-SAX columns are uniquely suited for the purification of therapeutic oligonucleotides, utilizing common buffers like Tris-HCl with organic modifiers for gRNA separations, or strong basic conditions for separating oxidation impurities of thiolated oligonucleotides.

Unlike traditional silica and small-pore chemistries, PL-SAX has the pH and temperature stability—paired with optimal pore size—required to separate various oligonucleotide sizes and modifications.

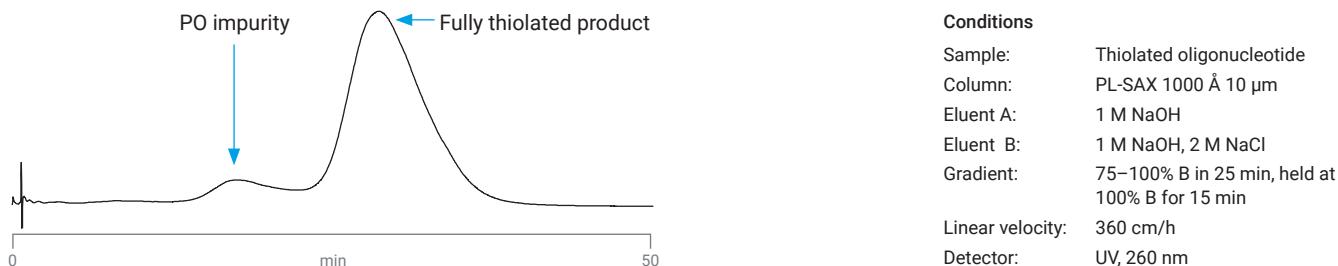


Figure 11. Using PL-SAX columns, a high-pH eluent can be used to separate a fully thiolated oligonucleotide from an impurity where thiolation is incomplete. The strong anion exchange functionality on a chemically inert polymeric matrix provides charge differentiation even in 1 M NaOH.

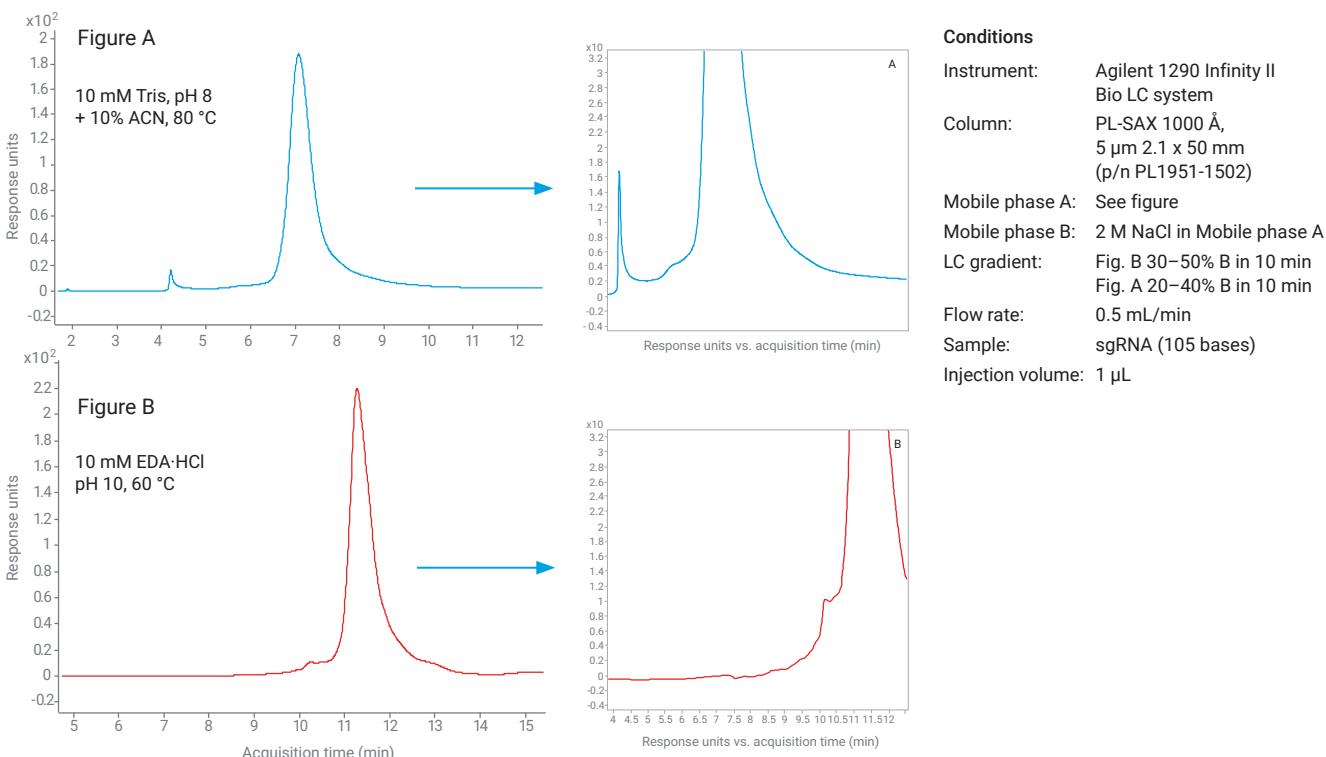


Figure 12. HPLC separation of sgRNA (105 bases) using PL-SAX 1000 Å, 5 µm 2.1 x 50 mm (p/n PL1951-1502). Figure A demonstrates separation of sgRNA utilizing standard 10 mM Tris, pH 8 + 10% ACN at elevated temperature. Figure B utilizes decrease in temperature and high pH to achieve increased resolution with a pH 10 ethylenediamine hydrochloride buffer EDA*HCl.

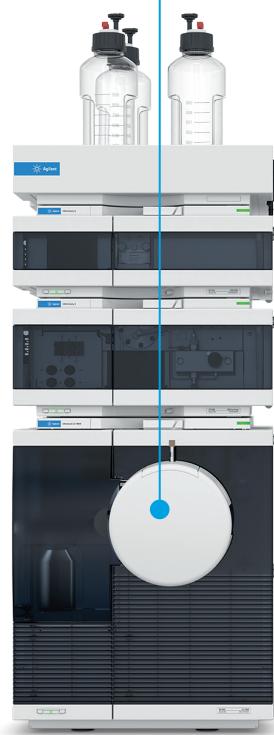
The benchmark in purification

The 1290 Infinity II Preparative LC system embodies the next generation of instrumentation for preparative LC, delivering the maximum in purification, instrument, and laboratory efficiency. Set the benchmark in your laboratory and boost your daily throughput.

Automated calculation of focused gradient profiles for each target compound helps you to achieve highest purity and recovery.

Our wide range of detectors for fraction triggering—including mass-based detection—ensure that you achieve maximum purity and recovery.

The 1290 Infinity II Preparative Open-Bed Sampler/Collector optimizes bench space. Benefit from the built-in fraction delay calibration of all InfinityLab fraction collectors.



Agilent Load & Lock columns

Ideal for routine, large-quantity purification, Agilent Load & Lock columns exhibit excellent packed bed stability and enhanced flow distribution. Combined with Agilent purification systems, these columns offer maximum efficiency at higher flow rates and delivery pressures, meeting the high-throughput demands of pilot-scale purification.

Easy to set up, easy to use

Within a few minutes, you can pack or unpack your column with any commercially available material—even in hazardous environments. Even better, the column and packing station are combined into a convenient stand, making them easy to move.

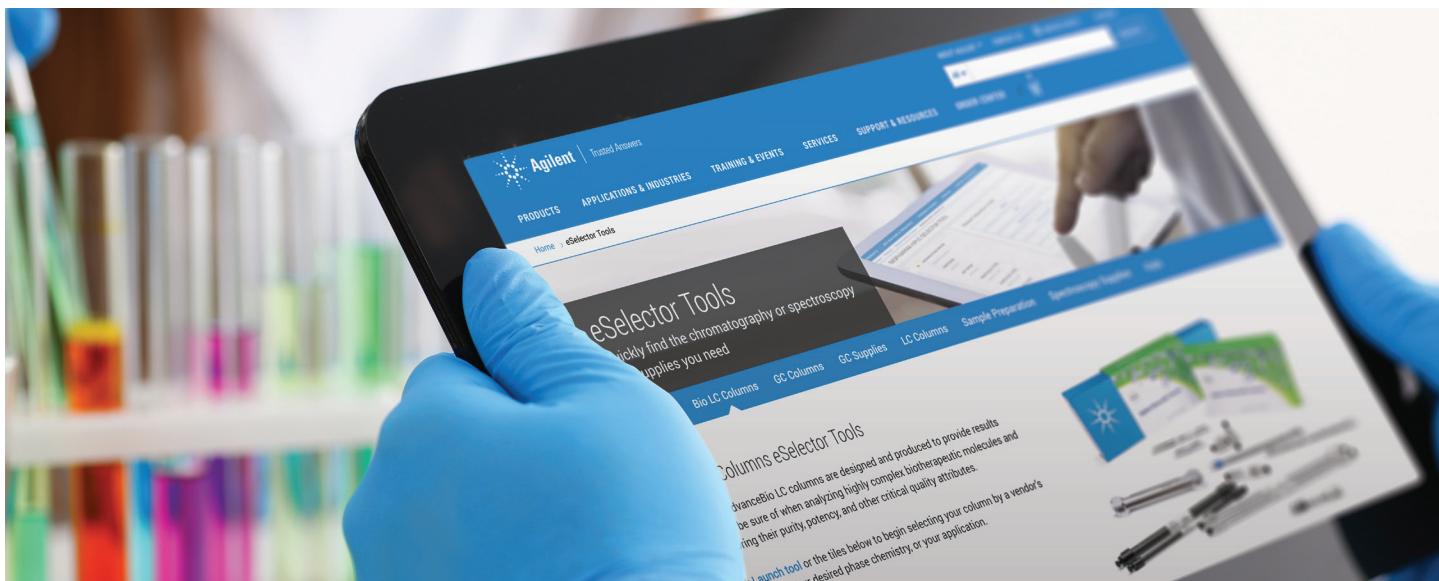
High performance on a large scale

Agilent Load & Lock columns are available with inside diameters from 1 to 3 inches (27 to 75 mm). They also offer both dynamic axial compression (DAC) and static axial compression (SAC).

Axial compression is used in the column packing process to compress the sorbent particles into a tightly packed, void-free bed for high-performance purifications. DAC constantly compresses the packed bed during use. With SAC, the column is first compressed by a plunger, which is held in position by a locking mechanism.



Pack multiple columns into one mobile station, and use the unlocking feature to deploy the packed columns anywhere in your lab.



Ordering Information

Agilent PL-SAX analytical columns

Dimensions (mm)	Particle Size (μm)	PL-SAX 1000 Å	PL-SAX 4000 Å
2.1 x 50	5	PL1951-1502	PL1951-1503
4.6 x 50	5	PL1551-1502	PL1551-1503
2.1 x 50	8	PL1951-1802	PL1951-1803
2.1 x 150	8	PL1951-3802	PL1951-3803
4.6 x 50	8	PL1551-1802	PL1551-1803
4.6 x 150	8	PL1551-3802	PL1551-3803
4.6 x 150	10	PL1551-3102	PL1551-3103
4.6 x 250	10	PL1551-5102	PL1551-5103
4.6 x 150	30	PL1551-3702	PL1551-3703
4.6 x 250	30	PL1551-5702	PL1551-5703

Agilent PL-SAX preparative columns

Dimensions (mm)	Particle Size (μm)	PL-SAX 1000 Å	PL-SAX 4000 Å
7.5 x 50	8	PL1151-1802	PL1151-1803
7.5 x 150	8	PL1151-3802	PL1151-3803
25 x 50	10	PL1251-1102	PL1251-1103
25 x 150	10	PL1251-3102	PL1251-3103
25 x 150	30	PL1251-3702	PL1251-3703
50 x 150	10	PL1751-3102	PL1751-3103
50 x 150	30	PL1751-3702	PL1751-3703
100 x 300	10	PL1851-2102	PL1851-2103
100 x 300	30	PL1851-3102	PL1851-3103

Agilent PL-SAX bulk media

Particle Size (μm)	Unit	PL-SAX 1000 Å	PL-SAX 4000 Å
10	100 g	PL1451-4102	PL1451-4103
	1 kg	PL1451-6102	PL1451-6103
30	100 g	PL1451-4702	PL1451-4703
	1 kg	PL1451-6702	PL1451-6703

Custom columns and bulk media

If you do not see the combination of pore/particle size and column dimensions or the bulk media quantity you are looking for in these tables, please contact your **local sales office**, who will assist you with our custom ordering process.

Agilent PLRP-S analytical columns

Size (mm)	Particle Size (μm)	PLRP-S 100 Å USP L21	PLRP-S 300 Å USP L21	PLRP-S 1000 Å USP L21	PLRP-S 4000 Å USP L21
4.6 x 250	8	PL1512-5800	PL1512-5801	PL1512-5802	
4.6 x 150	8	PL1512-3800	PL1512-3801	PL1512-3802	PL1512-3803
4.6 x 50	8		PL1512-1801	PL1512-1802	PL1512-1803
4.6 x 250	5	PL1512-5500	PL1512-5501		
4.6 x 150	5	PL1111-3500	PL1512-3501		
4.6 x 50	5	PL1512-1500	PL1512-1501	PL1512-1502	PL1512-1503
4.6 x 150	3	PL1512-3300	PL1512-3301		
4.6 x 50	3	PL1512-1300	PL1512-1301		
2.1 x 250	8		PL1912-5801		
2.1 x 150	8		PL1912-3801	PL1912-3802	PL1912-3803
2.1 x 50	8		PL1912-1801	PL1912-1802	PL1912-1803
2.1 x 250	5	PL1912-5500	PL1912-5501		
2.1 x 150	5	PL1912-3500	PL1912-3501		
2.1 x 50	5	PL1912-1500	PL1912-1501	PL1912-1502	PL1912-1503
2.1 x 150	3	PL1912-3300	PL1912-3301		
2.1 x 50	3	PL1912-1300	PL1912-1301		
1.0 x 50	8			PL1312-1802	
1.0 x 50	5	PL1312-1500			PL1312-1502
1.0 x 150	3	PL1312-3300			
1.0 x 50	3	PL1312-1300			
PLRP-S guard cartridges for 3.0 x 5.0 mm, 2/pk		PL1612-1801	PL1612-1801	PL1612-1801	PL1612-1801
Guard cartridge holder for 3.0 x 5.0 mm cartridges		PL1310-0016	PL1310-0016	PL1310-0016	PL1310-0016

Agilent PLRP-S prep to process columns

Size (mm)	Particle Size (μm)	PLRP-S 100 Å	PLRP-S 300 Å	PLRP-S 1000 Å	PLRP-S 4000 Å
100 x 300	30			PL1812-3102	PL1812-3103
100 x 300	15–20	PL1812-6200	PL1812-6201		
100 x 300	10–15	PL1812-6400	PL1812-6401		
100 x 300	10	PL1812-6100	PL1812-6101		
100 x 300	8	PL1812-6800	PL1812-6801		
50 x 300	8	PL1712-6800	PL1712-6801		
50 x 150	30			PL1712-3702	PL1712-3703
50 x 150	15–20	PL1712-3200	PL1712-3201		
50 x 150	10–15	PL1712-3400	PL1712-3401		
50 x 150	10	PL1712-3100	PL1712-3101	PL1712-3102	PL1712-3103
50 x 150	8	PL1712-3800	PL1712-3801		
25 x 300	15–20	PL1212-6200	PL1212-6201		
25 x 300	10–15	PL1212-6400	PL1212-6401		
25 x 300	10	PL1212-6100	PL1212-6101		
25 x 300	8	PL1212-6800	PL1212-6801		
25 x 150	30			PL1212-3702	PL1212-3703
25 x 150	10	PL1212-3100	PL1212-3101	PL1212-3102	PL1212-3103
25 x 150	8	PL1212-3800	PL1212-3801		
25 x 50	10			PL1212-1102	PL1212-1103

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