Technical Overview

# Generating Pure Oligonucleotides with PLRP-S

**Author**

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## Introduction

PLRP-S reversed-phase HPLC columns and media from Agilent deliver the pure oligonucleotides (oligos) required for genomics applications, including quantitative PCR, microarrays, and gene synthesis assays.

Key benefits include:

- Exceptional resolution and selectivity
- High purity levels
- Excellent column lifetime

## Exceptional performance

Agilent PLRP-S easily accommodates high temperatures for better selectivity and resolution between impurities and the target oligo. With PLRP-S stable polymeric reversed-phase material, oligos can be purified at temperatures up to 80 °C, while maintaining packed bed integrity.

PLRP-S is equally suited for purifying either trityl-off or trityl-on oligos. The outstanding resolution achieved with PLRP-S makes purification of even trityl-off oligos possible, which eliminates the extra steps required in trityl-on purifications. Since trityl-on failure sequences elute near the target product, trityl-off purifications have the added advantage of giving a cleaner separation of the target oligo.

As shown in Figure 1, PLRP-S is uniquely compatible with the higher temperatures that deliver superior resolution and selectivity

PLRP-S can be used to separate n-1 failure sequences, unincorporated dyes, and other unincorporated modifications from full-length target oligonucleotides.

| Conditions | |
|---|---|
| Sample | Poly(dT) 19-24 ladder |
| Column | PLRP-S 100Å 4.6 x 50 mm, 3 µm |
| Eluent A | 100 mM triethylammonium acetate (TEAA) |
| Eluent B | 100 mM TEAA in 25:75 acetonitrile:water |
| Flow Rate | 1.0 mL/min |

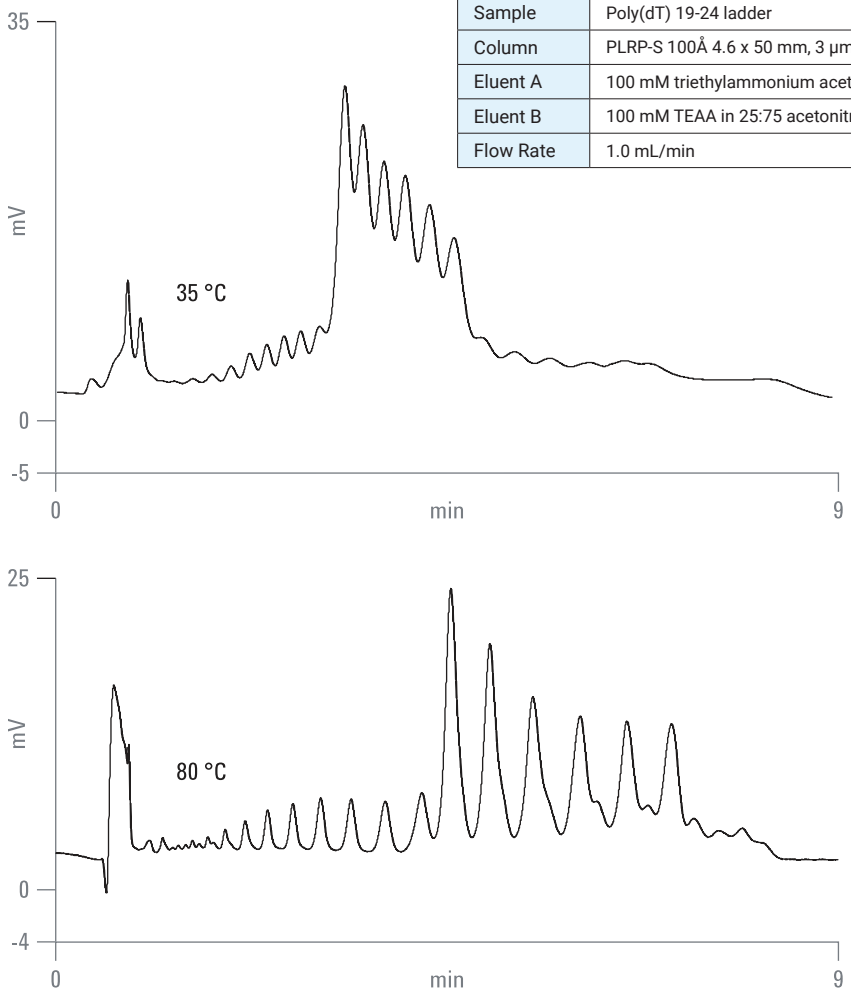


**Figure 1.** Reversed-phase separation of Poly(dT) 19-24 ladder at 35 and 80 °C.

## Cleaner oligonucleotides

The thermal stability of PLRP-S media is superior to that of silica-based material. Silica-based media has been demonstrated to break down at high temperatures over time, leading to sample contamination and a reduction in purity.

With PLRP-S stable polymer-based material, samples remain free of disrupted reversed-phase ligand and free of dissolved column media (see Figure 2).

## Increased column lifetime

PLRP-S rugged polymeric media is temperature stable up to 80 °C and chemically stable across the pH range. As a result, column lifetime is significantly improved, making PLRP-S the cost-effective choice.

## Experimentally proven

PLRP-S and three other oligonucleotide purification columns were analyzed for stability and longevity. Columns were subjected to 25-minute gradient cycles at the manufacturers' recommended temperatures. Resolution of a 29/30 mer sample was used to monitor column performance.

The performance of all of the test columns, except PLRP-S, fell below a usable level over the course of the study. As shown in Figure 3, PLRP-S column lifetime significantly exceeds that of leading silica-based columns because of its stable polymeric media.

At the end of the study, the head of each column was examined. As shown in Figure 4, only PLRP-S maintained its integrity, while the other columns exhibited voids from media breakdown.

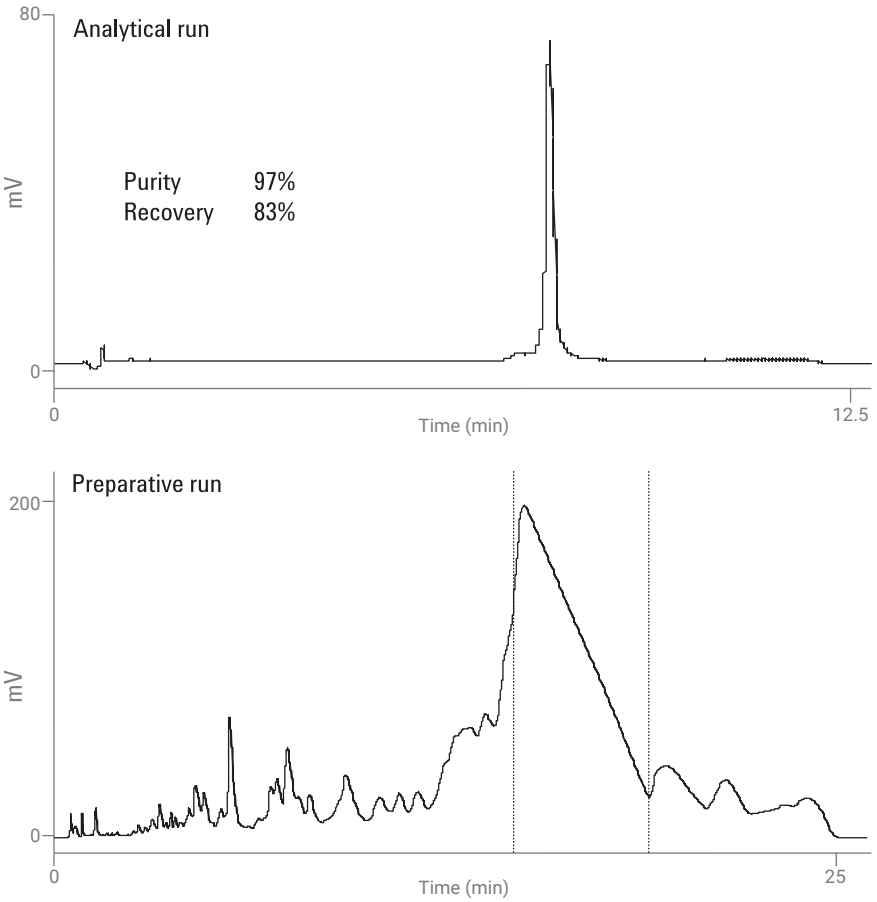


**Figure 2.** Purification of a 25mer trityl-off and analytical quantitation of fraction. Higher purity levels. 100 nmol purification: 25mer trityl-off, 4.6 × 50 mm PLRP-S column.

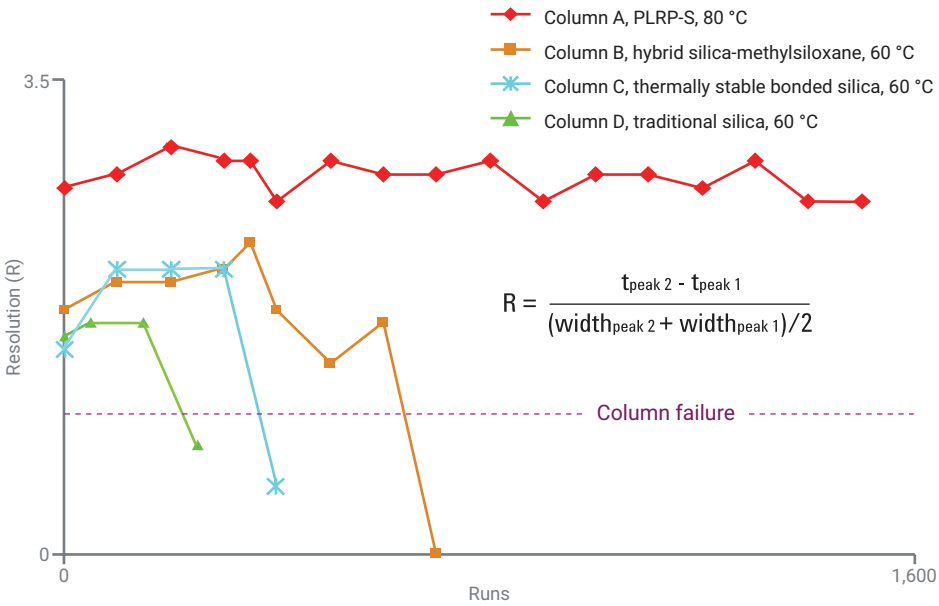


**Figure 3.** Column lifetimes at elevated temperature, plot of numbers of cycles versus resolution.


**Figure 4.** Column inlet photographs at the end of the stability trial. PLRP-S media remains intact at higher temperatures, keeping oligo samples free of dissolved media.

# Ordering information

## Agilent PLRP-S 100Å columns

| Column Dimensions | Column Sizes (Length) | | | |
|---|---|---|---|---|
| | 50 mm | 150 mm | 250 mm | 300 mm |
| 1.0 mm (id) | 1.0 × 50 mm | 1.0 × 150 mm | | |
| 3 µm | PL1312-1300 | PL1312-3300 | | |
| 2.1 mm (id) | 2.1 × 50 mm | 2.1 × 150 mm | | |
| 3 µm | PL1912-1300 | PL1912-3300 | | |
| 4.6 mm (id) | 4.6 × 50 mm | 4.6 × 150 mm | 4.6 × 250 mm | |
| 3 µm | PL1512-1300 | PL1512-3300 | | |
| 5 µm | | PL1111-3500 | PL1512-5500 | |
| 8 µm | | PL1512-3800 | PL1512-5800 | |
| 10 µm | | PL1512-3100 | PL1512-5100 | |
| 10 to 15 µm | | PL1512-3400 | PL1512-5400 | |
| 15 to 20 µm | | PL1512-3200 | PL1512-5200 | |
| 7.5 mm (id) | | | | 7.5 × 300 mm |
| 8 µm | | | | PL1112-6800 |
| 25 mm (id) | | 25 × 150 mm | | 25 × 300 mm |
| 8 µm | | PL1212-3800 | | PL1212-6800 |
| 10 µm | | PL1212-3100 | | PL1212-6100 |
| 50 mm (id) | | 50 × 150 mm | | 50 × 300 mm |
| 8 µm | | PL1712-3800 | | PL1712-6800 |
| 10 µm | | PL1712-3100 | | PL1712-6100 |
| 100 mm (id) | | | | 100 × 300 mm |
| 10 to 15 µm | | | | PL1812-6400 |
| 15 to 20 µm | | | | PL1812-6200 |

## Agilent PLRP-S 100Å bulk media

| Dimensions | Quantities | |
|---|---|---|
| | 100 g | 1 kg |
| 8 µm | | PL1412-6800 |
| 10 µm | PL1412-4100 | PL1412-6100 |
| 10 to 15 µm | PL1412-4400 | PL1412-6400 |
| 15 to 20 µm | PL1412-4200 | PL1412-6200 |
| 50 µm | PL1412-4K00 | PL1412-6K00 |

### Custom column and bulk media ordering

Columns and bulk media quantities not listed above can be ordered easily:

- For columns, indicate column dimensions, bonded phase type, particle size, and pore size.
- For bulk media, indicate quantity, bonded phase type, particle size, and pore size.
- Please fax your request to (302) 993-5354 or email it to cag_sales-na@agilent.com.
- Or you can place your column request at www.agilent.com/chem/customlccol and your bulk media request at www.agilent.com/chem/biohplcprep.

## Agilent chemistries: providing you confidence and control

Agilent's broad chemistries selection puts you in control of even the most difficult analyses. We manufacture columns and media that suit nearly every technique for small molecule, large molecule, and synthetic polymer analysis, enabling scaling from conventional 5 µm to "fast LC" sub-2 µm and superficially porous particles, and up to prep scale.

You can be confident that Agilent's meticulous end-to-end oversight of production delivers you the highest column consistency and performance. With more than 40 years of experience in the production of polymers and silica chemistries, our team is committed to the continuous development of new column advances, so you stay ahead of the curve with the technology that will make you the most productive.

You can count on Agilent to support you at every step. Agilent's infrastructure enables a delivery network that gets you what you need fast, anywhere in the world. That infrastructure also provides worldwide columns and chemistries technical support, as well as speedy problem resolution if you need it.

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DE44257.4690972222


Printed in the USA, March 10, 2021
5990-8187EN

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