BioProcess Chromatography

Purification Media For

- Peptides
- Oligonucleotides
- Proteins

Polymer Laboratories

Now a part of Varian, Inc.

NOTICE: This document contains references to Varian. Please note that Varian, Inc. is now part of Agilent Technologies. For more information, go to **www.agilent.com/chem.**

Agilent Technologies

Rigid Polymerics: The Future of Prep/Process Chromatography

Polymer Laboratories (PL), now a part of Varian, Inc., is a long-established manufacturer of specialist polymeric particles for chromatographic, pharmaceutical and diagnostic applications.

Since 1976, the Company has grown dramatically, and comprises specialists in polymers for chromatography, drug discovery, pharmaceutical and clinical applications. Technical staff include biopolymer and polymer scientists, chromatography specialists and chemical engineers.

The Company has progressively expanded its manufacturing facilities to ensure reliable customer supply. In 2001, the Company opened its new, state of the art, chemical engineering plant, with a capacity in excess of 4 M tonnes of high performance media, and established its place as one of the world's largest bulk media suppliers.

Polymer Laboratories is an international Company, with offices in the US, UK, The Netherlands, Germany and France. Elsewhere, PL products are supported by a technical worldwide distributor network.

Highest Quality, Highest Performance Resin

PL has been manufacturing polystyrene/divinylbenzene (PS/DVB) particles since 1976. The production of PL media and resin is tightly controlled and monitored within a stringent quality system, ensuring product reproducibility from batch to batch throughout the entire product range.

An innovative approach to product development has resulted in a range of world-class commercial and custom products spanning the synthesis, identification and purification of small to large biomolecules. PL's particle products include oligonucleotide and solid phase synthesis supports and clinical diagnostic particles, through to high performance separation media. PL's customers include leading global pharmaceutical and bioscience companies.

1976	PS/DVB particle production commences
1984	PLRP-S reversed phase media introduced
1986	PL manufactures preparative HPLC media
1986	PL-SAX strong anion exchange media introduced
1988	Diagnostic latex particles introduced
1992	Solid phase synthesis supports introduced
1997	ISO 9001 Accreditation
1999	PL manufactures process scale HPLC media
2002	Process scale bulk media is manufactured in new, state of the art, production facility
2005	Polymer Laboratories acquired by Varian, Inc.

Rigid Polymerics - The Future of Prep/Process Chromatography

PL's high performance particles are manufactured by controlled polymerization of styrene/divinylbenzene. The rigid macroporous styrene/divinylbenzene media is used for reversed phase chromatography, PLRP-S, and, after coating/chemical modification, as a strong anion exchanger, PL-SAX. The Company's proprietary production processes give complete control over pore and particle size and surface chemistry, ensuring reproducibility from lot to lot. The optimized pore structure provides the chromatographic characteristics required for the purification of a wide range of biomolecules, from small peptides and oligonucleotides through to large proteins and DNA fragments.

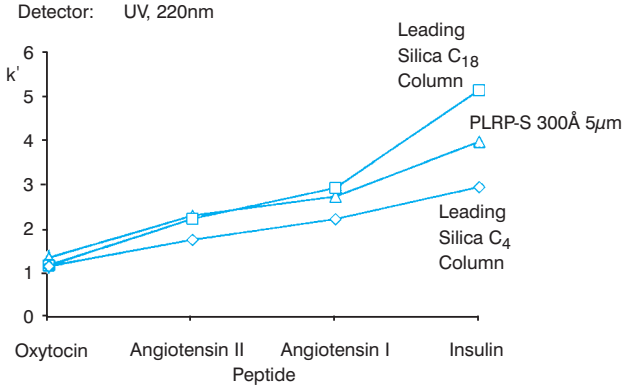
Parallel Performance

The chromatographic retention of PL's rigid polymeric particles is comparable to 'softer' polymers and silica based particles. Using PL media, there is no costly method development involved in switching to these more robust prep/process materials - *simply* improved process economics.

In reversed phase chromatography, it would be expected that the hydrophobicity of alkyl-bonded silica would differ significantly from that of the aromatic groups present on the surface of a polystyrene/divinylbenzene matrix. However, the observed capacity factor *k'* (calculated from the formula $k' = (t_R - t_0)/t_0$ where t_0 is the retention time for a non-sorbed solute) for four peptides on leading C_{18} and C_4 silica columns and PLRP-S indicates the similarity of the interaction. The retention characteristics of the PLRP-S column provide excellent performance for a wide range of peptides.

Comparison of Retention Characteristics - PLRP-S and Commercial Silica-Based Packings

Columns: 250x4.6mm
Eluent A: 0.1% TFA in 20% ACN : 80% Water
Eluent B: 0.1% TFA in 50% ACN : 50% Water
Gradient: 0-100% B in 15 mins
Flow Rate: 1.0ml/min
Detector: UV, 220nm



PL-SAX media is used for anion exchange chromatography over a wide pH range (up to *pH 12*), and exhibits retention characteristics similar to conventional soft polymers.

Column/media lifetimes *cannot*, however, be similarly compared: the long term chemical and physical stability of PL's polymeric media ensures reproducible resolution with greatly extended column lifetimes, even in excess of two years.

Rigid Macroporous Polymer Particles From PL Provide *Improved Economics* Through

Chemical, Physical & Thermal Stability →	***Unsurpassed Column Lifetimes***
Chemical & pH Stability →	***Enhanced Selectivity & CIP***
Optimized Pore Structure →	***Increased Capacity***
No Leachables →	***Cleaner Product***

Solvent Compatibility

Exceptionally low swell is achieved via the high crosslinked density of the polymer structure, ensuring complete gradient compatibility with the widest range of organic modifiers. Using PLRP-S, it is not necessary to dedicate columns to particular organic modifiers as transfer between modifiers can be rapidly achieved.

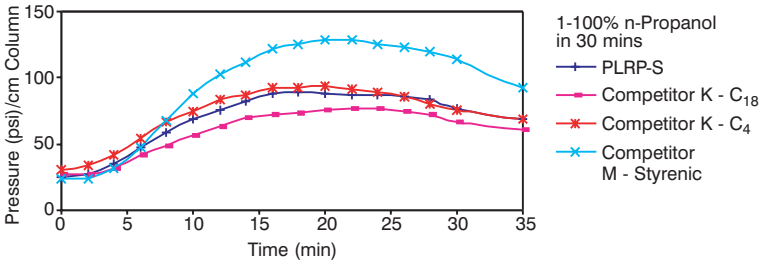
Typical Mobile Phase Modifiers

Acetonitrile	Ethanol	n-Propanol
Acetone	Isopropanol	Tetrahydrofuran
Dimethylsulfoxide	Methanol	

Gradient Stability

With the PLRP-S media, operating pressure and changing pressure through an aqueous/organic gradient is comparable to silica based materials, and considerably less than observed with a competitor's polymeric media. No change in swell is detected as the organic content of the eluent changes.

Pressure Through Aqueous/Organic Gradient



Chemical and pH Stability

Enhanced Selectivity for Improved Loading

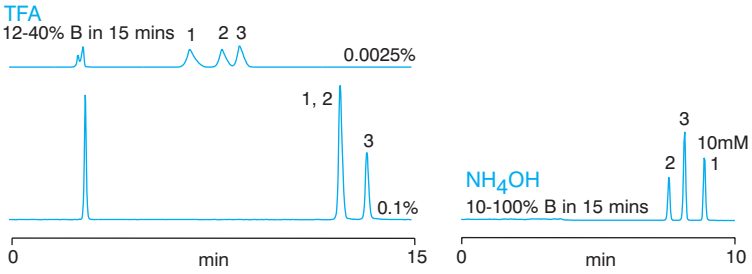
PLRP-S and PL-SAX media from Polymer Laboratories is stable over the pH range 1-14. The apparent hydrophobicity, net ionic charge, of the target molecule and/or impurities can be manipulated by control of pH to enhance selectivity.

Traditionally, peptide separations have been performed at acidic pH, within the limited pH stability range of silica-based materials. The absence of silanols with PLRP-S media, as would be present with silica based materials, allows low levels of TFA to be used for reversed phase separations. Alternatively, separations can be performed under neutral and basic pH, improving the resolution and loading of the purification.

Effect of pH on Peptide Selectivity

Column: PLRP-S 100Å 10-15µm, 250x4.6mm
Flow Rate: 1.0ml/min
Detector: UV, 220nm

KEY
1. Angiotensin III
2. Angiotensin II
3. Angiotensin I



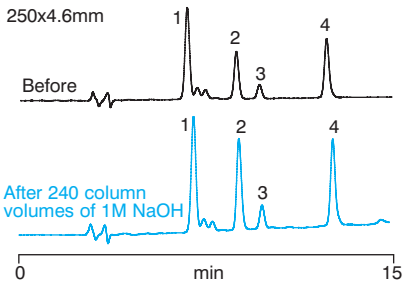
Cleaning in Place

PL's media is chemically robust and can withstand extremely aggressive sanitizing/cleaning protocols. Media can be cleaned in a packed column (CIP), or in bulk, using a range of solubilizing agents such as sodium hydroxide to ensure unsurpassed column/media lifetimes.

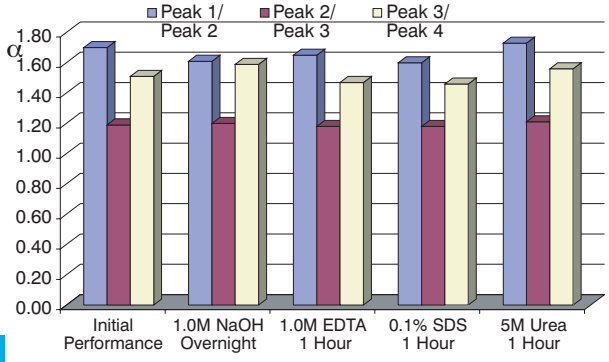
1M Sodium Hydroxide Stability

Column: PLRP-S 300Å 10-15µm, 250x4.6mm
Eluent A: 0.1% TFA in Water
Eluent B: 0.1% TFA in ACN
Gradient: 20-50% B in 15 mins
Flow Rate: 1.0ml/min
Detector: UV, 220nm

KEY
1. Oxytocin
2. Angiotensin II
3. Angiotensin I
4. Insulin



Peptide Selectivity after CIP Procedures



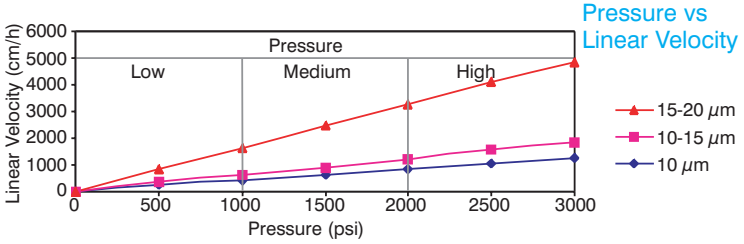
Physical and Thermal Stability

Physical Stability

PL particles are physically robust and stable to pressures of up to 6000 psi. They can be packed in high performance, high pressure column hardware, and also in DAC systems. There is no compression of the particle when operated under HPLC conditions of pressure and flow rate.

To maximize throughput, it is essential that high linear velocities are used for the purification but that the operating pressure is compatible with the equipment being used. PL therefore produces three particle sizes to cover the range of system operating pressures.

Pressure vs Linear Velocity

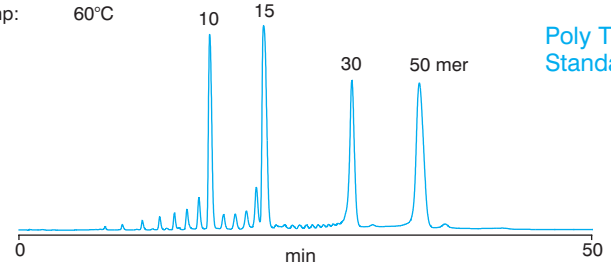


Thermal Stability

PL's media is thermally stable for separations which require elevated temperatures, such as oligonucleotide separations which are routinely run at 60ºC.

Column: PL-SAX 1000Å 8µm, 50x4.6mm
Eluent A: 93% 0.1M TEAA, pH 8.5 : 7% ACN
Eluent B: 93% 0.1M TEAA, 1M Ammonium chloride, pH 8.5 : 7% ACN
Gradient: 0-40% B in 10 mins, 40-70% B in 14 mins, 70-100% B in 25 mins
Flow Rate: 1.5ml/min
Temp: 60°C

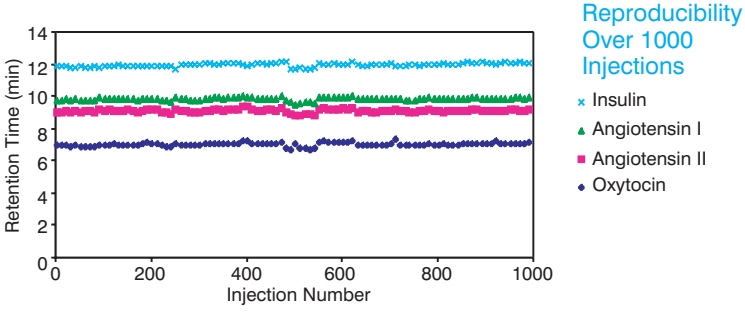
Poly T - Standard



Unsurpassed Column Lifetimes

The rigidity of PL's polymeric particles prevents compaction and ensures packed bed stability under biotherapeutic purification conditions of high linear velocity. The advantages to the User are faster run times for higher throughput and an extended number of cycles between column repackings, thereby improving process economics.

Reproducibility Over 1000 Injections



The retention times, resolution and column efficiency remain unchanged after 1000 injections of the standard peptide mixture.

Optimized Pore Structure

Maximum Throughput

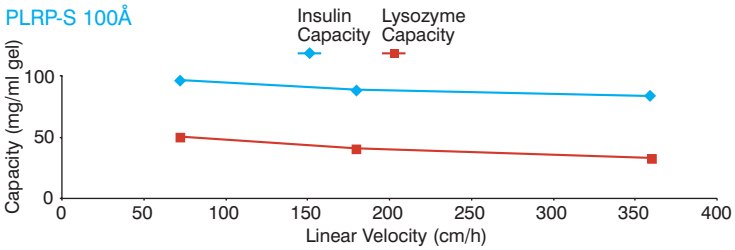
To maximize throughput it is essential that the chromatographic media has maximum loading capacity under the purification conditions used. Loading will be influenced by the pore size, pore size distribution and pore volume of the media as these parameters determine the available surface area. The size of the molecule to be purified will determine the minimum pore diameter of the media to be used.

Capacity mg/mL CV

	PLRP-S		*PL-SAX*	
	100Å	*300Å*	*1000Å*	*4000Å*
Insulin	95	60	-	-
Lysozyme	55	45	-	-
BSA	5	25	80	35
Oligonucleotide	72	54	-	-

The optimized pore structure of the media ensures excellent mass transfer characteristics. Feedstock can be loaded at high linear velocity with minimal reduction in dynamic capacity.

Capacity Dependency on Linear Velocity



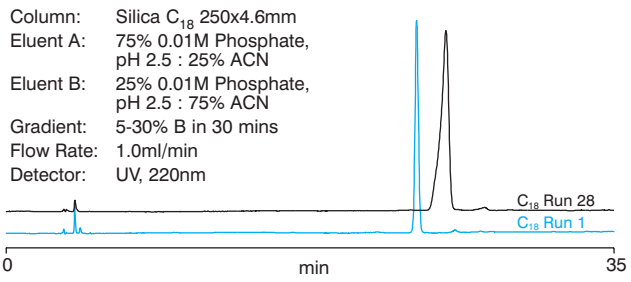
No Leachables

Stable Polymeric Particles - Cleaner Bio-Product

PL's stringent production process removes all manufacturing by-products, producing the cleanest media possible.

PL's media does not suffer from base particle degradation or bonded phase stripping, unlike conventional silica reversed phase material. The result is no contamination of your product with silica and/or alkyl ligands, and guaranteed run to run reproducibility.

Silica Degradation with a Basic Peptide Solution



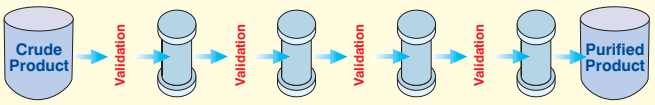
A new silica C_{18} column was used to determine the purity of the fractions collected from a pH 9 synthetic peptide purification run. After only 28 injections into a buffered low pH system, the column retention characteristics had changed and efficiency had been lost. No deterioration was observed when a PLRP-S column was used.

Vastly Improved Process Economics

Unit volume costs for PL's polymeric media are comparable with the leading process reversed phase silica or 'softer' polymerics *and*, with PLRP-S and PL-SAX media, you have the additional benefits of:

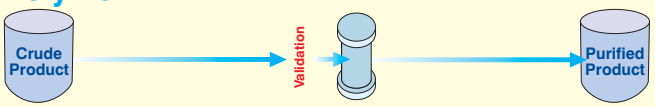
Features	Benefits
Cleaner media means no leachables Chemical stability means no particle degradation or bonded phase stripping	*No product contamination*
Range of pore sizes for maximum loading Optimized pore structure permits high feed rates Mechanical stability allows use of high linear velocities Chemical stability enables pH to be used as a variable to enhance selectivity and improve loading	*Increased throughput*
Batch to batch reproducibility gives column to column consistency Chemical stability gives reproducibility throughout the column lifetime	*Consistent chromatography*
Rigid particles give packed column stability Chemical stability allows aggressive CIP for 'dirty' feedstocks and column regeneration No production downtime due to column depacking/ repacking/validation	*Significantly longer column lifetimes*

Silica



Typically, up to 4 silica columns may be needed to produce the same amount of purified product as 1 PLRP-S column.

Polymer



IMPROVED PROCESS ECONOMICS REDUCED PRODUCTION COST

Synthetic Peptides
Reversed Phase

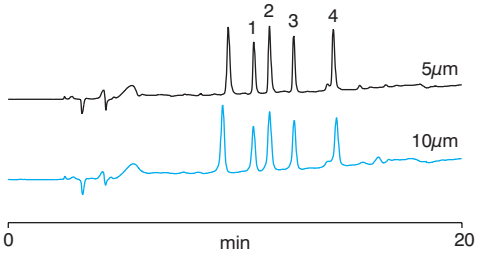
- Exceptional Selectivity
- Ease of Scale Up, µg ➔ Multi kg
- High Efficiency at every scale, Analytical through Process
- Chromatography across the entire pH range

Tripeptide Analysis & Purification

This analysis of four synthetic tripeptides demonstrates equivalent retention characteristics for 5µm analytical and 10µm prep/process media.

Columns: PLRP-S 100Å 5µm and PLRP-S 100Å 10µm
Eluent A: 0.1% TFA in 95% Water
Eluent B: 0.1% TFA in 50% ACN
Gradient: 0-100% B in 20 mins
Linear Velocity: 360cm/h
Detector: UV, 220nm

KEY
1. Phe-Ile-Gly
2. Phe-Ile-Ala
3. Phe-Val-Ala
4. Phe-Ile-Val



- ***Baseline resolution of four closely related tripeptides illustrates excellent selectivity***

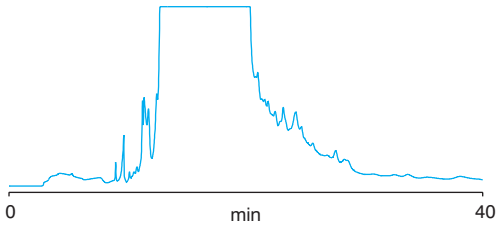
High Capacity/High Speed

To maximize throughput, the chromatographic media must be able to operate at high linear velocity and with high sample loading. The optimized pore size and high mechanical stability of the PLRP-S media is ideally suited to provide high throughput and hence improved process economics.

Purification of a Synthetic Peptide

Using PLRP-S 100Å 10µm media to maximize productivity, synthetic peptides can be subjected to a purification regime under overload conditions.

Sample: Phe-Ile-Val Tripeptide
Column: PLRP-S 100Å 10µm
Eluent A: 0.1% TFA in 95% Water
Eluent B: 0.1% TFA in 50% ACN
Gradient: 0-100% B in 20 mins
Linear Velocity: 360cm/h
Detector: UV, 220nm



- ***95% purity was obtained with an 86% yield in a single purification run***

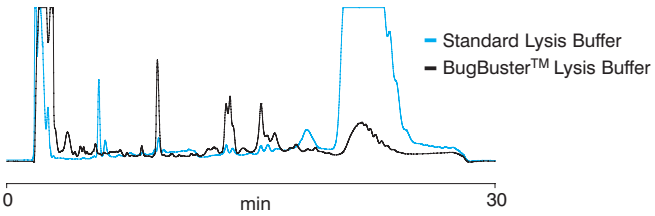
Recombinant Peptides/Proteins
Reversed Phase

- Optimized 300Å Pore Size
- High Resolution for Polishing
- Inert Media Resistant to Fouling
- Depyrogenation with 1M NaOH

E-coli Lysates

E-coli lysates can be injected directly onto a PLRP-S column after filtration to remove cell debris/insoluble material. The elution profiles for a standard lysis buffer and the commercial BugBuster™ lysis buffer are shown below, illustrating the complexity of the sample matrix.

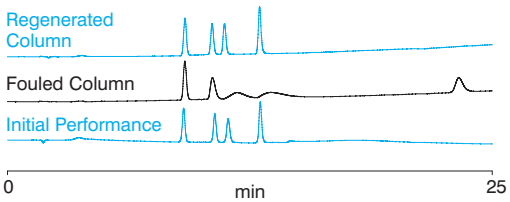
Column: PLRP-S 300Å 10µm
Eluent A: 0.1% TFA in 95% Water
Eluent B: 0.1% TFA in 80% ACN
Gradient: 0-100% B in 20 mins
100% B for 5 mins
Linear Velocity: 360cm/h
Detector: UV, 220nm



CIP Regimes (Clean in Place)

The *E-coli* lysate is an extremely complex matrix containing a wide range of cellular components. To assess the robustness of PLRP-S media, 200µl aliquots of the filtered crude lysate were injected.

After 95 injections of the crude lysate, some deterioration of column performance was observed: pressure had increased, plate count was lost and a deterioration in the separation of a standard peptide mixture was observed. A strict CIP regime was followed using 1M NaOH/ACN and THF/ACN. As the separation of four synthetic peptides below illustrates, after CIP had been performed, the column pressure came down, the plate count was restored and the peptide separation was as for the 'new' column.



- ***The column is completely regenerated***

Synthetic Oligonucleotides

Anion Exchange

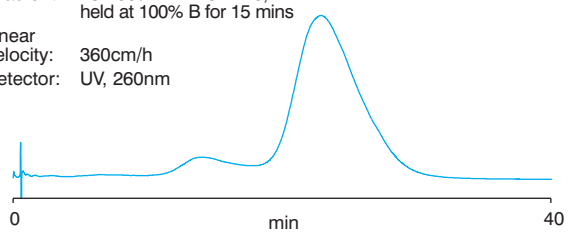
- High Performance Chromatography at High pH
- Excellent Temperature Stability
- Large Pore Size Optimized for Large Biomolecules
- High Resolution

Use of high pH has been shown to improve chromatography where an oligonucleotide is prone to self association/aggregation.

Thiolated Oligonucleotide

Using PL-SAX material, a high pH eluent can be used to separate a fully thiolated oligonucleotide from an impurity where thiolation is incomplete.

Column: PL-SAX 1000Å 10µm
Eluent A: 1M NaOH
Eluent B: 1M NaOH, 2M NaCl
Gradient: 75-100% B in 25 mins, held at 100% B for 15 mins
Linear Velocity: 360cm/h
Detector: UV, 260nm



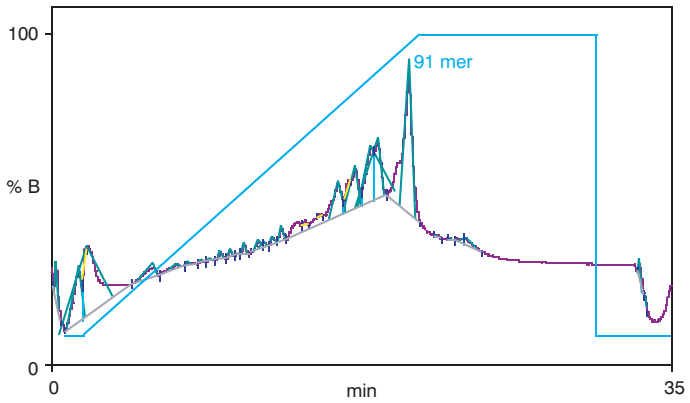
- *The strong anion exchange functionality on a chemically inert polymeric matrix gives charge differentiation even in 1M NaOH*

Purification of a Large Oligonucleotide

It is essential for many applications that the full length oligo(n) is purified from the failure sequences, including n-1.

For large oligomers, wide pore PL-SAX media is required to provide maximum load with no mass transfer restrictions.

Column: PL-SAX 1000Å 8µm
Eluent A: 93% 0.1M TEAA, pH 7 : 7% ACN
Eluent B: 93% 0.1M TEAA, 3.24M Ammonium acetate, pH 7 : 7% ACN
Gradient: 0-100% B in 20 mins
Flow Rate: 1.5ml/min
Temp: 60°C
Detector: UV, 290nm



- *Using PL-SAX, the large 91 mer oligo is purified from the failure sequences*

Reversed Phase

Trityl On / Trityl Off

- The optimized pore sizes of the reversed phase PLRP-S media (100Å and 300Å) make it ideal for oligonucleotide purification.

- For separation of trityl on/trityl off oligonucleotides, the high available surface area and homogeneous clean surface of the PLRP-S media give maximum load and recovery.

Ion Pair Chromatography

- For dynamic ion pair chromatography, the small particle size PLRP-S provides high resolution separations.

20 mer Oligonucleotide Capacity on PLRP-S 100Å Media with TBAB Ion Pairing Agent

Temperature	*Loading*
60ºC	72 mg/ml
Ambient	63 mg/ml

PL Expertize

Polymeric Particle Production

Polymer Laboratories, now a part of Varian, Inc., has been manufacturing particles for the leading bioscience/pharma/clinical companies since 1976, with one of the largest production capabilities worldwide

Smart Products for Synthesis, Analysis & Purification
Since 1976

Support Activities

PL offers full, confidential support for its preparative and process media.

- Choice of media
- DMF
- RSF

The team of scientists specialising in biopurification will assist with your initial application and separation optimization, through process development and scale up to production implementation.

Custom Media

- Particle Size Optimization
- Pore Size Optimization
- Surface Functionalities

PL has complete control over the manufacturing process for its full range of prep/process media. This means that where no commercially available material with the required characteristics for a process scale purification exists, PL will work with the client to design and manufacture a suitable material. This could involve increasing the load and/or changing the surface chemistry/functionality to improve selectivity and resolution.

Ordering Information

PLRP-S 100Å/300Å Columns	10µm		10-15µm		15-20µm	
Dimensions mm	Part No. 100Å	Part No. 300Å	Part No. 100Å	Part No. 300Å	Part No. 100Å	Part No. 300Å
PREP/PROCESS EVALUATION COLUMNS						
150x4.6	PL1512-3100	PL1512-3101	PL1512-3400	PL1512-3401	PL1512-3200	PL1512-3201
250x4.6	PL1512-5100	PL1512-5101	PL1512-5400	PL1512-5401	PL1512-5200	PL1512-5201
150x25	PL1212-3100	PL1212-3101				
300x25	PL1212-6100	PL1212-6101				
150x50	PL1712-3100	PL1712-3101	PL1712-3400	PL1712-3401	PL1712-3200	PL1712-3201
300x50	PL1712-6100	PL1712-6101	PL1712-6400	PL1712-6401	PL1712-6200	PL1712-6201
300x100	PL1812-6100	PL1812-6101	PL1812-6400	PL1812-6401	PL1812-6200	PL1812-6201

5µm and 8µm PLRP-S columns and media also available.
Request our Analytical Chromatography Catalog or visit our website at www.polymerlabs.com

PLRP-S 100Å/300Å Media			
Particle Size	Qty	Part No. 100Å	Part No. 300Å
10µm	10g	PL1412-2100	PL1412-2101
	100g	PL1412-4100	PL1412-4101
	1 kg	PL1412-6100	PL1412-6101
10-15µm	10g	PL1412-2400	PL1412-2401
	100g	PL1412-4400	PL1412-4401
	1 kg	PL1412-6400	PL1412-6401
15-20µm	10g	PL1412-2200	PL1412-2201
	100g	PL1412-4200	PL1412-4201
	1 kg	PL1412-6200	PL1412-6201
50-70µm	10g	PL1412-2600	PL1412-2601
	100g	PL1412-4600	PL1412-4601
	1 kg	PL1412-6600	PL1412-6601

For multi-kilo applications,
bulk discounts and call-off orders,
please call to discuss your requirements

PL-SAX 1000Å/4000Å Columns	8µm		10µm	
Dimensions mm	Part No. 1000Å	Part No. 4000Å	Part No. 1000Å	Part No. 4000Å
50x4.6	PL1551-1802	PL1551-1803		
150x4.6	PL1551-3802	PL1551-3803		
50x7.5	PL1151-1802	PL1151-1803		
150x7.5	PL1151-3802	PL1151-3803		
50x25			PL1251-1102	PL1251-1103
150x25			PL1251-3102	PL1251-3103
150x50			PL1751-3102	

PL-SAX 1000Å/4000Å Media			
Particle Size	Qty	Part No. 1000Å	Part No. 4000Å
10µm	10g	PL1451-2102	PL1451-2103
	100g	PL1451-4102	PL1451-4103
	1 kg	PL1451-6102	PL1451-6103

Contact the UK office for details of your local distributor

Your local distributor is:

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2. *C1 Domain of Protein Kinase C, with phorbol ester bound. Courtesy of Erik Hom, Tami Marrone and Andrew McCammon, University of California at San Diego.*

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