PRODUCT MANUAL

ProSwift™ WAX

DIONEX

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

PRODUCT MANUAL

FOR

PROSWIFT™ WAX MONOLITHIC COLUMN

(PEEK-lined/Stainless Steel, 4.6 x 50mm, P/N 064294)

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GUIDE TO IMPORTANT INFORMATION

SAFETY ***Safety information can help prevent bodily harm.***

WARNING ***Warning information can help prevent equipment harm.***

CAUTION ***Caution information can help prevent problems.***

NOTE ***Note information can help with tips for improved use.***

1. INTRODUCTION

1.1. Morphology of ProSwift Anion Exchange Monoliths

ProSwift™ WAX-1S monolithic columns are specifically designed to provide high-resolution and high efficiency separations of proteins and oligonucleotides.

ProSwift media are based on polymeric monoliths prepared by an *in situ* polymerization process. They are a new generation of separation media which are uniquely designed and engineered for separation of biomolecules. The monolith is a single cylindrical polymer rod containing an uninterrupted, interconnected network of through pores which are also called channels. Its unique morphology, pore structure and pore size distribution offer the optimum performance for separation of proteins, large peptides, oligonucleotides, and other biomolecules.

The morphologies of the ProSwift WAX-1S (P/N 064294) monoliths are shown in Figure 1. The monoliths consist of aggregates of globules shaped like cauliflower. The open spaces among the large aggregates are the large flow-through channels allowing flow without high back pressure. The spaces among the smaller globules are the open or through-pores allowing fast access of the samples to the functionalized surface of the media. The mass transfer of the samples is primarily driven by convective flow through these open pores instead of much slower molecular diffusion. These pores are large enough for even large molecules to flow through freely. Most of the small globules are engineered to be less than 500 nm. Therefore, the path lengths for mass transfer through these small globules are much shorter than the path lengths in conventional bead-based chromatographic phases. In addition, the globules are essentially non-porous based on BET measurements and SEM examinations. Diffusion-controlled mass transfer is eliminated because these globules are non-porous. This is in contrast to porous beads where diffusion-controlled mass transfer predominates.

In summary, flow-through pores, short mass transfer paths, and non-porous globules are characteristics unique to ProSwift monolith morphology. These monolith features enable much faster analyte mass transfer compared to porous beads.

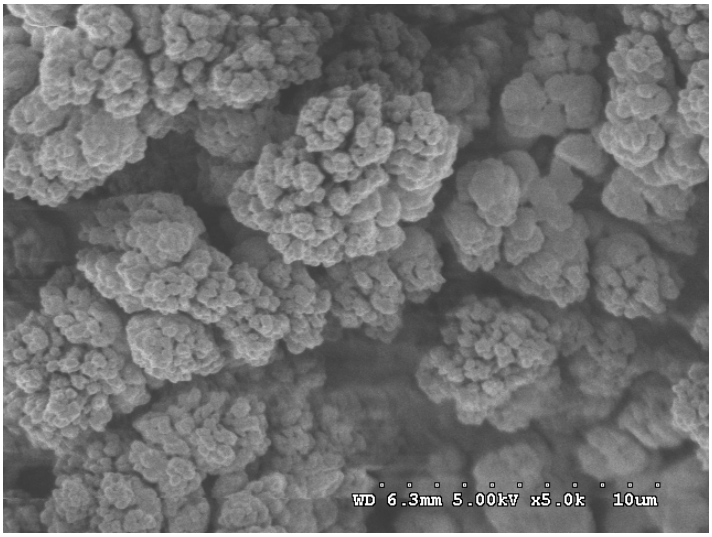


Figure 1
SEM Image of the ProSwift WAX-1S Monolith

1.2. Backpressures and Pore Size Distributions

ProSwift monoliths have very high permeability. The pore volume is about 60% of the column volume, which is much higher than the porous beads. There are two types of pores: large pores (approximately a micron or larger) for eluent to flow through and the small pores (ten to hundreds of nanometers) for most of the separations to take place. The modal pore size for the WAX-1S is about 1750 nm, as shown in Figure 2. These large pores allow the eluent to flow though with moderate back pressure, and allow higher flow rates for faster separations. The backpressure generated at different flow rates of ProSwift WAX-1S is shown in Figure 3. At any given flow rate, the backpressures generated on ProSwift columns are typically lower than those of bead-based analytical columns.

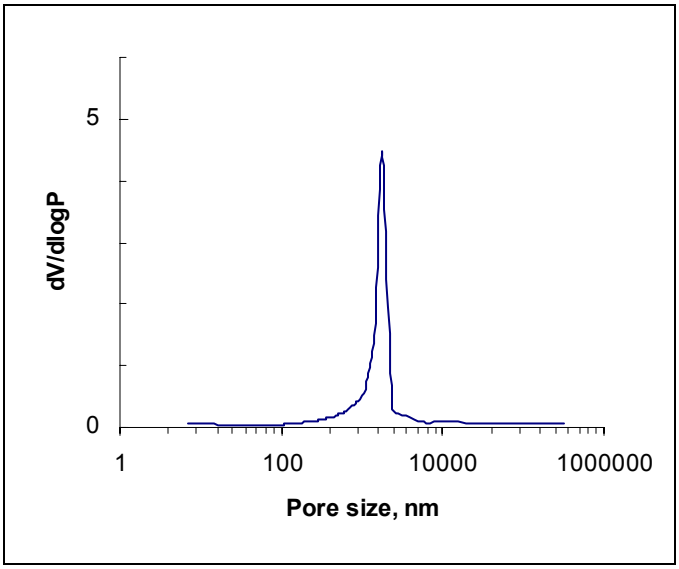


Figure 2
Pore Size Distribution of Proswift WAX-1S by Mercury Porosimetry

System:	Summit HPLC
Column:	ProSwift WAX-1S
Eluent:	0.02M Tris to pH 8 with HCl

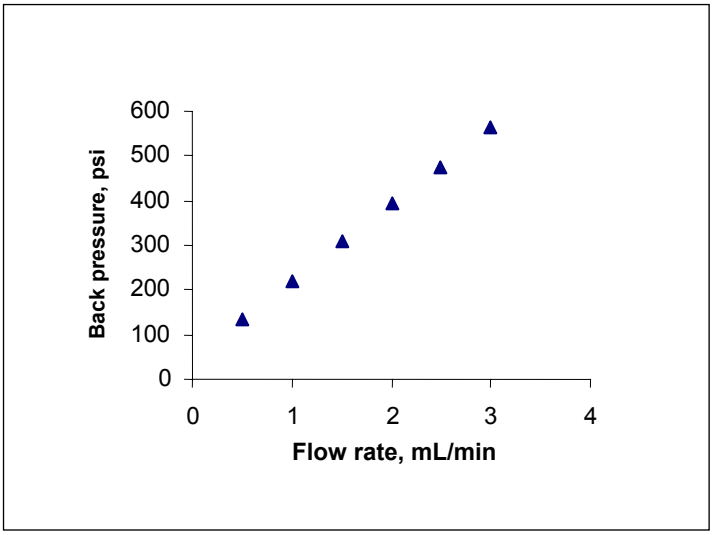


Figure 3
Flow Rate and Backpressure Correlations for ProSwift WAX-1S

1.3. Resolution and Speed of Separation

As described in section 1.1, the uniquely designed morphology of ProSwift monoliths allows fast analyte mass transfer. This property minimizes band broadening upon flow rate increases, thus resulting in higher resolution than conventional beads over a wide range of flow rates. This is especially true for large molecules, whose diffusivities are much lower than small molecules. This provides the benefits of non-porous beads. In combination with their low back pressure, ProSwift monoliths offer excellent separations at low and high flow rates, which improve productivity.

1.4. Loading Capacity

ProSwift monolith surfaces are irregular as shown in Figure 1. This produces loading capacity comparable to porous beads. This optimized morphology supplies the best features of non-porous beads and porous beads: high resolution and high capacity: "The best of both worlds!"

Figure 4a shows the relative capacities of the nonporous DNAPac PA100 4x50 mm column and the ProSwift WAX-1S 4.6 x 50mm monolith. The figure shows the two columns to give comparable peak shapes when 0.5 µg or less is injected. However, when more sample is injected, the peak width of the DNAPac column increases while that of the ProSwift column remains stable up to ~ 50 µg. At a peak width of ~ 0.5 min, the ProSwift column exhibits 10-15 times the capacity of the DNAPac.

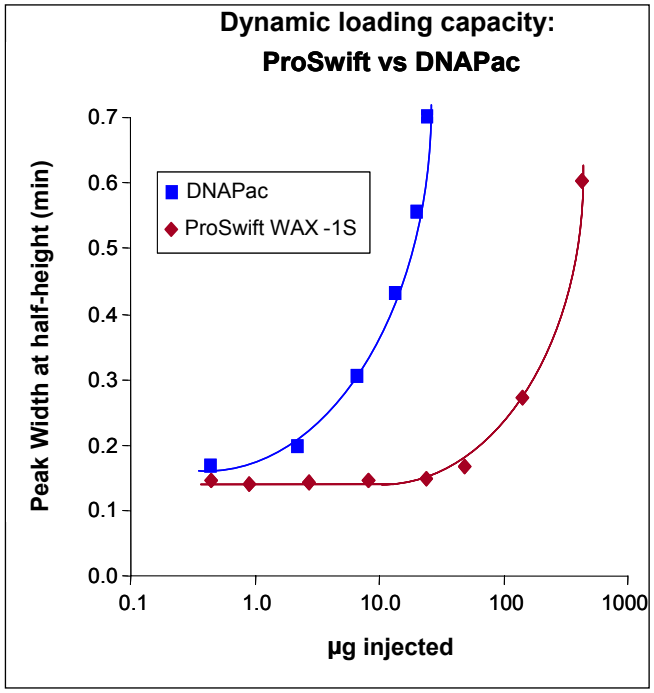


Figure 4a
Comparison of Loading Capacity of ProSwift Columns with a Leading DNA Analysis Column: DNAPac PA100

Figure 4b compares the loading capacity of these columns. When 120 µg of the 25-base oligonucleotide (with and without the trityl protecting group) was injected on each column at pH 8 using a salt gradient. The ProSwift monolith delivers 10-14 fold more capacity than the DNAPac column. This produces analytical resolution with purification capacity as shown.

System:	Dionex ICS 2500
Columns:	ProSwift WAX-1S, DNAPac PA100 4x50 mm
Flow:	1.0 mL/min
Eluent:	0.02M Tris to pH 8 with HCl
	300-925 mM NaCl in 15 min
Detection:	Absorbance at 260 nm
	(0.4mm path length cell)
Samples:	25mer mixed-base oligonucleotide in tritylated and detritylated forms.

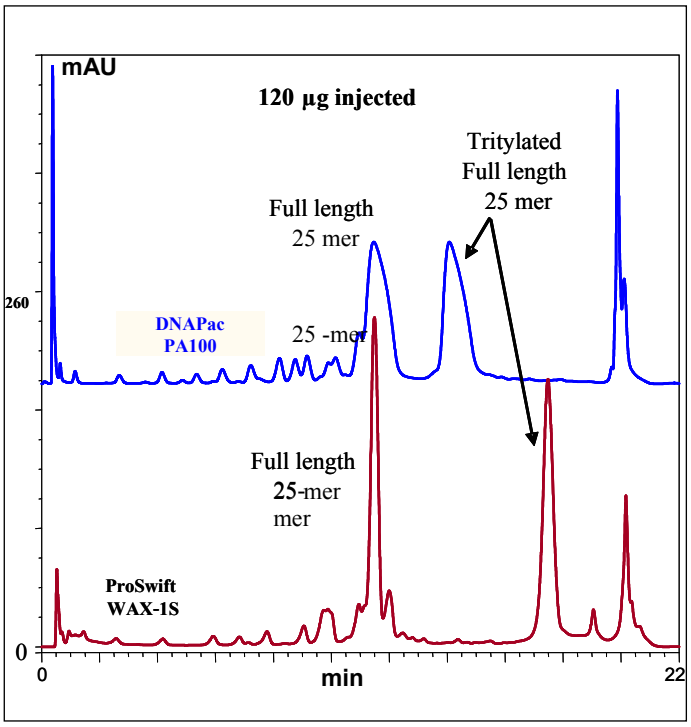


Figure 4b
Comparison of Peak Width at Half Height vs
Sample Load of ProSwift WAX-1S and DNAPac PA100 4x50

The protein loading capacity of Ovalbumin on ProSwift WAX-1S Monolith is shown in Figure 4c. Up to 1120 µg ovalbumin was loaded on to the column and could be separated.

Column:	ProSwift WAX-1S, 4.6 x 50 mm
Flow:	1.0 mL/min
Eluent:	A. 10 mM Tris (pH 7.6) B. 10 mM Tris + 1 M NaCl (pH 7.6)
Detection:	UV at 280 nm
Sample:	Ovalbumin (14 mg/mL each)
Inj. Volume:	5 µL to 80 µL
Gradient:	2-50%B in 15 min.

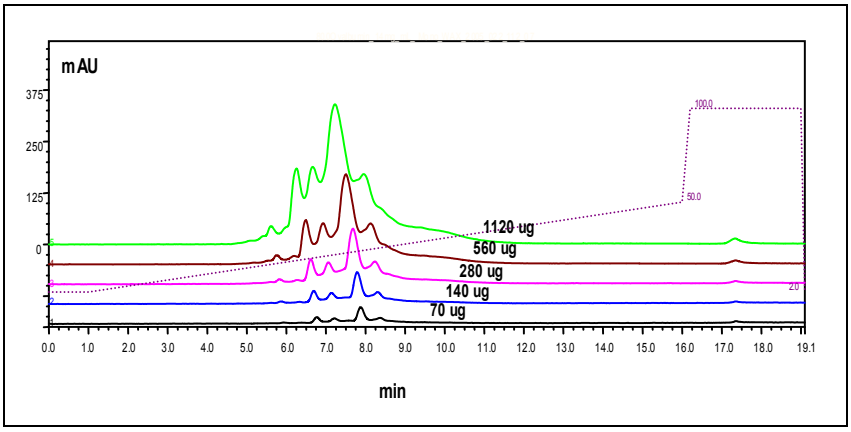


Figure 4c
Protein Loading Capacity of ProSwift WAX-1S Column

1.5. Robustness and Run Stability

Durability and robustness are ProSwift characteristics. The ProSwift monoliths exhibit stability and reproducibility even after hundreds of runs. Figure 5 shows that ProSwift WAX -1S column retained high resolution over 200 cycles of operation and was assessed by injecting ovalbumin intermittently.

Column:	ProSwift WAX 1S (4.6 x 50 mm)
Flow rate:	1.0mL/min
Eluents:	A. 10 mM Tris (pH 7.6)
	B. 10 mM Tris + 1 M NaCl (pH 7.6)
Gradient	0 to 50% B in 5 min.
Detection:	UV at 214 nm
Sample:	Ovalbumin (2.0 mg/mL)
Inj. Volume:	5 µL

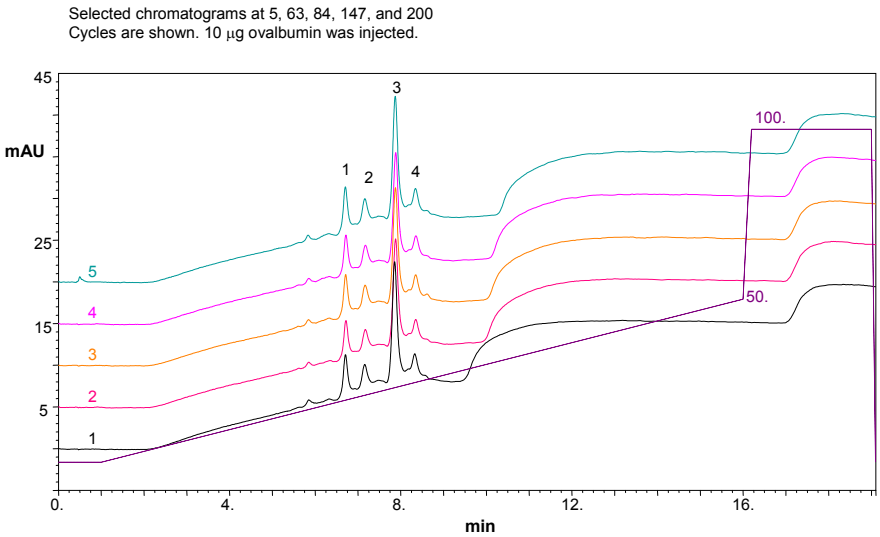


Figure 5
Ruggedness and Stability of ProSwift WAX-1S column

1.6. Batch-to-Batch Reproducibility

ProSwift anion exchangers are manufactured by a patented *in situ* manufacturing process which has the least number of variables affecting the reproducibility when compared to other technologies. This manufacturing process includes a single step polymerization followed by a single step surface modification. It does not require additional sieving, coating, multiple surface modification and packing processes. Therefore, batch-to-batch reproducibility is excellent for the monoliths.

Column:	ProSwift WAX-1S (4.6 x 50 mm)
Flow rate:	2.0mL/min
Eluents:	A. 10 mM Tris (pH 7.6)
	B. 10 mM Tris + 1 M NaCl (pH 7.6)
Gradient	0 to 75% B in 9 min.
Detection:	UV at 280 nm
Sample:	1. Ovalbumin (1.0 mg/mL) 2. Trypsin Inhibitor (1.0 mg/mL) 3. Insulin Chain A (0.25 mg/mL)
Inj. Volume:	10 µL

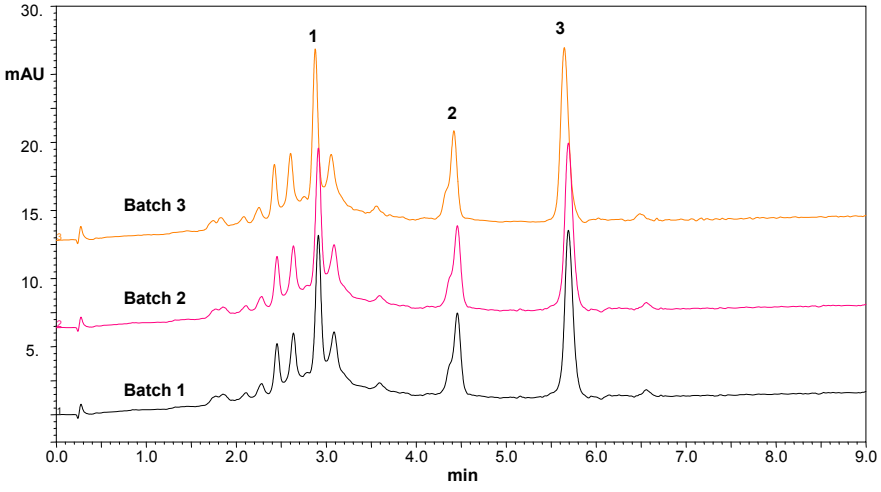


Figure 6
Batch-to-Batch Reproducibility of ProSwift WAX-1S Column

1.7. pH Stability

The ProSwift WAX-1S monolith has a polymethacrylate support with tertiary amine functional groups on the surface. The column is stable with treatment of 1 M NaOH and 0.1 M HCl in the regeneration process. It is stable at pH 2 to 12 under operating conditions for long periods.

1.8. Temperature Stability

Anion exchange phases produced with methacrylate polymer are stable at ambient temperatures from pH 2 to 12 using normal separation conditions. Temperature stability decreases at elevated pH. The ProSwift WAX-1S column tolerates temperatures to 80 °C for short periods, and is quite stable at temperatures up to 70 °C at pH from 6 to 8. At pH between 8.0 to 9.5, the maximum operating temperature is recommended to decrease linearly to ambient temperature.

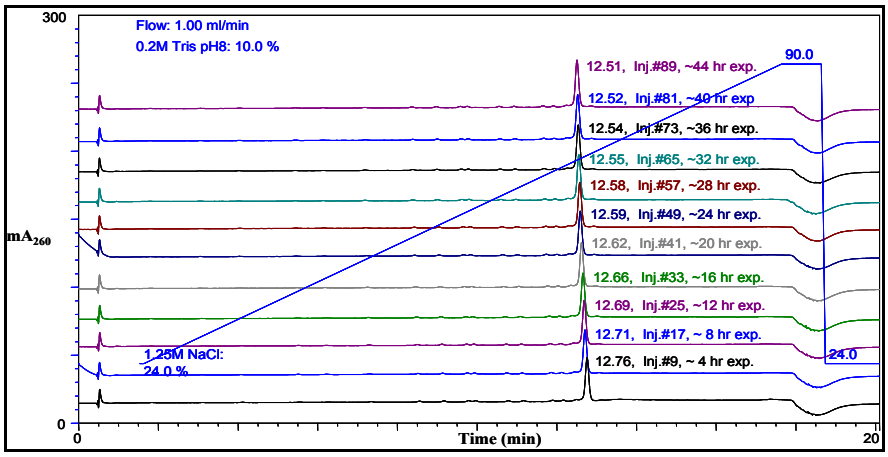


Figure 7
Retention of a mixed-base 25mer oligonucleotide on the ProSwift WAX-1S monolith at 70 °C

Oligonucleotide peak shape on the ProSwift WAX-1S monolith is shown to be reproducible during a 44 hour exposure to 70 °C. Retention, which diminished by only 0.25 min during the test, is within acceptable limits.

1.9. Scalability

The patented manufacturing process and column design of Dionex ProSwift monolithic anion exchange columns provide excellent scalability of the products. Figure 9 shows that the analytical size column was scaled to more than 300 times larger column resulting in essentially identical chromatograms.

Column:	ProSwift WAX-1S
Flow rate:	3.0 & 30 mL/min
Eluents:	A. 10 mM Tris (pH 7.6)
	B. 10 mM Tris + 1 M NaCl (pH 7.6)
Gradient	0-50% B for 10 column volumes
Detection:	UV at 280 nm
Sample:	1. Myoglobin (1.2 mg/mL) 2. Conalbumin (2,9 mg/mL) 3. Ovalbumin (2.1 mg/mL) 4. Trypsin Inhibitor (2.0 mg/mL)
Inj. Volume:	20 µL & 6 mL

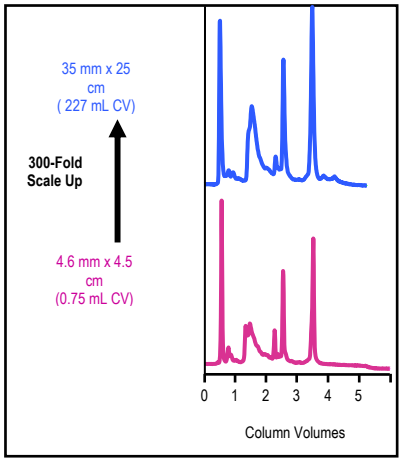


Figure 8
Comparison of resolutions of ProSwift analytical size column (0.7 mL CV) with large preparative size columns (227 mL CV)

1.10. Summary of Characteristics of ProSwift Monolithic Anion Exchanger

The following table summarizes the general characteristics of the ProSwift monolithic media.

Characteristics of ProSwift™ Monolithic Technology			LC Properties
Porous Structure	Flow-through pores	Fast mass transfer driven by convective flow	High efficiency
	No interstitial volume	Fast mass transfer due to short flow path	High efficiency
	Large pore & High permeability	High flow velocity with low back pressure	Fast separation
	Non-porous globules	Minimize diffusion controlled mass transfer within pores	High resolution
	Porous structure	Much higher surface area than non-porous beads	High capacity
	Chemically bonded aggregates	Rigid polymer structure due to chemical bonding between the aggregates	Excellent run-to-run stability
Manufacturing Process	Single-step in situ polymerization	Minimum number of reaction variables • No sieving steps • No coating steps • No multiple derivatization steps • No packing steps	Excellent batch to batch reproducibility
	Solution polymerization	Broad choice of monomers & Direct copolymerization of hydrophilic monomers	High selectivity High recovery
	Temperature programmed polymerization	Avoid heat transfer problems in scaling up process	Excellent scalability

1.11. Specifications

Parameter	ProSwift WAX-1S
Column Dimension	4.6 x 50mm
Base Matrix Material	Polymethacrylate
Surface Chemistry	Tertiary amine (diethylaminoethyl [DEAE])
Protein Binding Capacity per mL of Polymer	18 mg/mL BSA
Bed Height	44 mm
Bed Volume (Ml)	0.73 mL
pH Range	2 -12
Recommended Flow Rate	0.5 – 1.5 mL/min
Recommended Operating Pressure	< 500 psi (3.45 bars, 3.45 Mpa)
Maximum Flow Rate	2 mL/min
Recommended Temperature Range	Ambient at pH 2-12, 70 °C at pH 6-8
Maximum Pressure	600 psi (41 Bar, 4.1 Mpa)

2. INSTALLATION

2.1. System Requirements
ProSwift monoliths are designed to operate with standard HPLC systems that include inert gradient pumps, flow paths, and injection valve materials.

2.2. System Void Volume
Tubing between the injection valve and detector is recommended to be ≤ 0.010" I.D. PEEK tubing. Minimize the length of all liquid lines, especially the tubing between the column and the detector. The use of larger diameter and/or longer tubing may decrease peak efficiency and peak resolution.

2.3. Operational Parameters

pH Range:	pH = 2 - 12
Temperature Limit:	Up to 70 °C at pH 8.0 (lower at pH > 8.0)
Pressure Limit:	600 psi
Organic Solvent Limit:	100% acetonitrile if required for cleaning.
Detergent Compatibility:	Nonionic, cationic detergents.
Typical Buffer and Salts:	Tris, AMP, Ammonium, Sodium and Potassium salts of chloride, and perchlorate or acetate.

2.4. Eluent Limitations
The ProSwift anion exchange columns are compatible with typical eluents such as sodium or potassium chloride or sulfate salts in Tris, phosphate or acetate buffers, up to their limit of solubility. Use of organic solvents in the eluent may be helpful for very hydrophobic analytes, but is typically unnecessary. If you employ solvents, test their solubility with the salt-containing eluents prior to use. Some combinations of eluent salts and organic solvents are not miscible.

NOTE

Do not use anionic detergents because the detergents irreversibly bind to the substrate.

2.5. Chemical Purity Requirements
Obtaining sensitive, consistent, and accurate results requires eluents that are free of impurities. Chemicals, solvents and deionized water used to prepare eluents must be the highest purity available. Low trace impurities and low particle levels in eluents will extend the life of your ion exchange columns and system components. Dionex cannot guarantee proper column performance when the quality of the chemicals, solvents, and water used to prepare eluents is substandard.

2.5.1. Inorganic Chemicals
Always use reagent grade or better inorganic chemicals to prepare eluents. Whenever possible, use inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity. These chemicals will detail the purity with an actual lot analysis on each label.

When using solvents, HPLC Grade products or equivalent should be used to prepare eluents.

2.5.2. Deionized Water
Deionized water used to prepare eluents should be Type I Reagent Grade Water with a specific resistance of ≥ 18 megohm-cm. Ensure that it is free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Bottled HPLC-Grade Water from Burdick & Jackson is acceptable, but most other bottled water contains an unacceptable level of ionic impurities.

2.6. Eluent Preparation

2.6.1. Adjusting the pH of the Eluent

Add all eluent electrolytes to eluent solutions before adjusting the pH. To make sure that the pH reading is correct, calibrate the pH meter prior to use. Stir the solutions during adjustment and employ temperature correction. Take care to ensure the accuracy of the pH electrode when using Tris buffers because some electrodes give erroneous results with Tris.

2.6.2. Filtering the Eluent

To extend the lifetime of your column as well as your HPLC pump, filter all eluent buffers using a 0.2 µm membrane filter to remove insoluble contaminants from the eluents.

2.6.3. Degassing the Eluent

Before use, degas the eluents. Degassing can be done either by using Dionex pump degas functions as described in the pump manuals or by placing the eluent reservoir in a sonic bath and drawing vacuum on the filled reservoir with a vacuum pump for 5 minutes while operating the sonic bath.

3. OPERATION

3.1. QuickStart for ProSwift WAX-1S

I. Preparation:

a. Eluent Preparation
The following eluents are recommended, but the column may be used with any eluents suitable for your analysis. Typically Eluent A is a low salt buffer and Eluent B is a high salt buffer.
Eluent A: 0.01 M Tris •HCl buffer, pH 7.6
Eluent B: 1 M NaCl in 0.01 M Tris •HCl buffer, pH 7.6

b. Column Installation
Install the column on the LC instrument in the correct flow direction.

WARNING

Sudden increases in flow rates may damage the column.

Always increase the flow rate slowly using a linear flow gradient or stepwise flow increments.

If your eluent composition generates back pressure in excess of the maximum operation pressure, adjust the flow rate to ensure the backpressure of the column is less than the recommended maximum operation pressure.

c. Flow Rate Start-Up (Ramping)
Using a linear flow gradient increase the flow rate of Eluent A from 0.00 to your desired flow rate in 1 minute or by increasing the flow in stepwise increments of ≤0.50 mL/min every 30 seconds.

II. Column Conditioning:

a. Cleaning of Storage Solution:
- Run a 15 mL (20 column volumes) binary gradient from 100% A to 100% B at your desired flow rate.
- Pump another 15 mL (20 column volumes) 100% B through the column.

b. Column Equilibration:
- Equilibration from 100% B to your starting eluent composition should include at least 1 minute reverse gradient to initial conditions.
- Pump this eluent composition through the column for at least 15 mL (20 column volume).

III. Storage:

a. For short-term storage, <3 days, store the column in your initial buffer compositions.

b. For long-term storage, >3 days, use the following solution to avoid microbial growth on the column.
- 0.05 M NaCl
- 0.1% NaN_3
- 0.01 M Tris.HCl at pH 7.6

3.2. Increase Flow Rate

Establishing flow rate: Slowly increase the flow rate from less than 0.5 mL/min to the desired level (up to 2 mL/min in test chromatogram) over 1-2 minutes.

CAUTION

Sudden extreme increases in flow rates may damage the column. To prolong column life, avoid immediate and dramatic increases in the flow rate.

3.3. Sample Preparation

For best results, dissolve the samples in the initial run buffer or in pure deionized water. The salt concentration should be determined so the column is not overloaded by the injected sample.

Generally for protein analysis, loading of no more than 1 mg protein is recommended on the ProSwift WAX monolith, although, more can be loaded for purification purposes.

For oligonucleotide analyses, Dionex recommends loading no more than 120 µg, although more can be loaded for oligonucleotide purification (see Section 4.3.5).

If the sample contains particulates, filter it through a 0.2 µm syringe filter before loading it on to the column.

3.4. Column Equilibration

Before performing a run after storage, wash and equilibrate the column using protocols described in QuickStart (Section 3.1). When switching to a different buffer type, use an eluent volume of 20 times the column volume (~ 15 mL for 4.6 mm x 5.0 cm column) to ensure the monolith is well equilibrated.

4. APPLICATIONS

4.1. Elution Profiles on a ProSwift WAX-1S Anion-Exchange Column

Figure 9 shows the separation of protein mixture on ProSwift WAX-1S column. The protein mixture contained myoglobin, conalbumin, ovalbumin (contains two phosphorylation sites that result in closely related variants) and trypsin inhibitor.

Column:	ProSwift WAX-1S (4.6 x 50 mm)
Flow rate:	1.5 mL/min
Eluents:	A. 10 mM Tris (pH 7.6) B. 10 mM Tris + 1 M NaCl (pH 7.6)
Detection:	UV at 214nm
Sample:	4 protein mixture (1mg/mL each)
Inj. Vol:	20 uL
Gradient:	2-50%B in 10 min.
Order of elution:	1. Myoglobin 2. Conalbumin 3. Ovalbumin 4. Trypsin Inhibitor

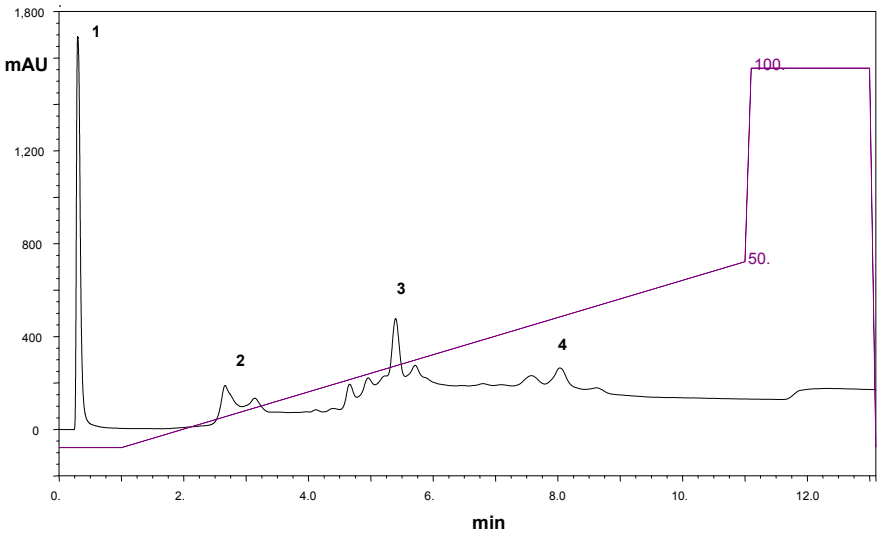


Figure 9
Separation of a Protein Mixture on ProSwift WAX-1S Monolith

4.2. Separation of Pancreatin

Pancreatin is a complex sample containing a variety of proteases (protein digesting enzymes like trypsin and pepsin), amylases (carbohydrate digesting enzymes), and lipases (fat digesting enzymes). Pancreatin was separated on the WAX column into an unbound and a bound fraction. Bound fraction contained several well resolved peaks.

Column:	ProSwift WAX-1S (4.6 x 50 mm)
Flow rate:	1.0 mL/min
Eluents:	A. 10 mM Tris (pH 7.6) B. 10 mM Tris + 1 M NaCl (pH 7.6)
Detection:	UV at 214nm
Sample:	Pancreatin (21mg crude protein suspended in 1mL H_2O, centrifuged and the clear supernatant used)
Inj. Vol:	10 uL
Gradient:	2-50%B in 15 min.

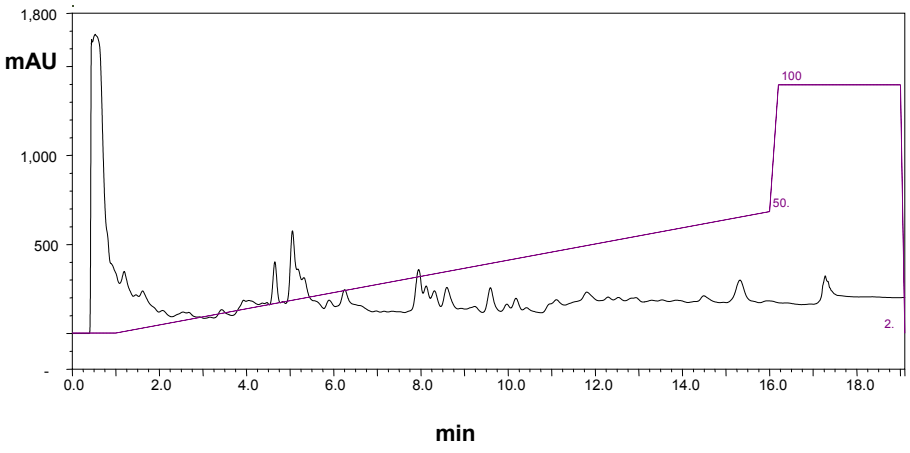


Figure 10
Separation of a Pancreatin on ProSwift WAX-1S Monolith

4.3. Oligonucleotide Separations

4.3.1. Effect of Oligonucleotide (ON) Length on Retention

Homopolymer oligonucleotides (phosphorylated deoxy-Adenyl oligos) elute in order of length (Figure 11). However, base composition also influences retention, so identical length mixed-base oligonucleotides with different base compositions may be fully resolved. Here, oligonucleotides from 12 to 30 bases long are fully resolved, and those from 40 to 60 bases are at least partially resolved in this 35 minute curved gradient of NaCl, at 1.0 mL/min.

System:	Dionex ICS 2500
Column:	ProSwift WAX-1S
Flow:	1.0 mL/min
Gradient:	0.02M Tris to pH 8 with HCl
	400-625 mM NaCl in 35 min, Curve 4
Detection:	Absorbance at 260 nm
Sample:	20 µL Phosphorylated Poly dA oligonucleotides 12-30 and 40-60 bases long.
Sample concentrations:	1 - 8 µg/mL each ODN.

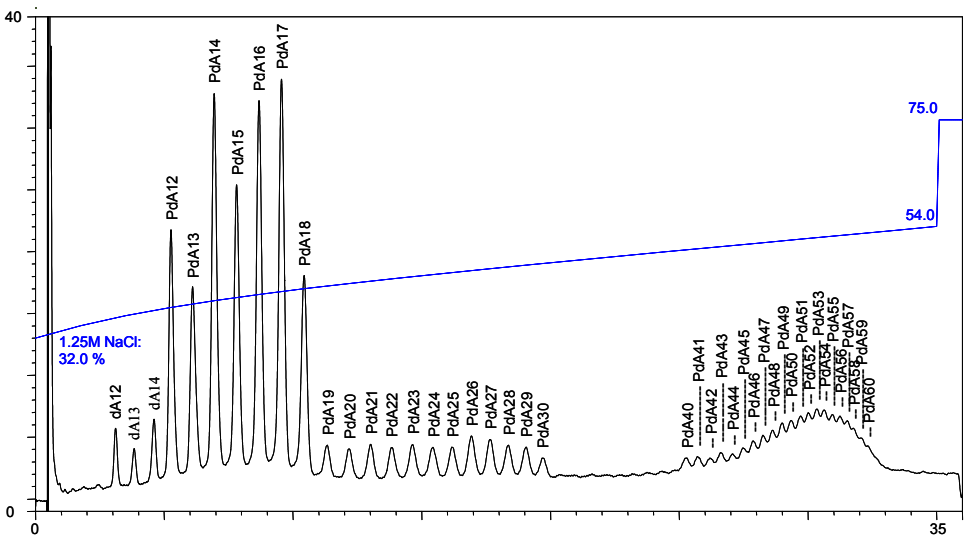


Figure 11
Elution of PdA Oligonucleotides on ProSwift WAX
400-625 mM NaCl in 35 min, 20 mM Tris pH8, 30 °C, Curve 4

4.3.2. Effect of pH on Oligonucleotide Retention

The ProSwift WAX is a weak anion exchanger, so its net charge diminishes as the pH rises above pH 8. As the net charge on the phase diminishes so does retention. In Figure 12, elution of two 25-base ONs is shown to occur earlier at pH 8.0 than pH 6.5, and significantly earlier at pH 9.5 than at pH 8.0.

System:	Dionex ICS 2500
Column:	ProSwift WAX-1S
Flow	1.0 mL/min
Gradient:	0.02M Tris to pH 8 with HCl
	338-825 mM NaCl in 25 min
Detection:	Absorbance at 260 nm
Samples:	25 base oligonucleotides with base compositions of A7G6T7C4 (Dx78) and A5G6T9C5 (Dx79).

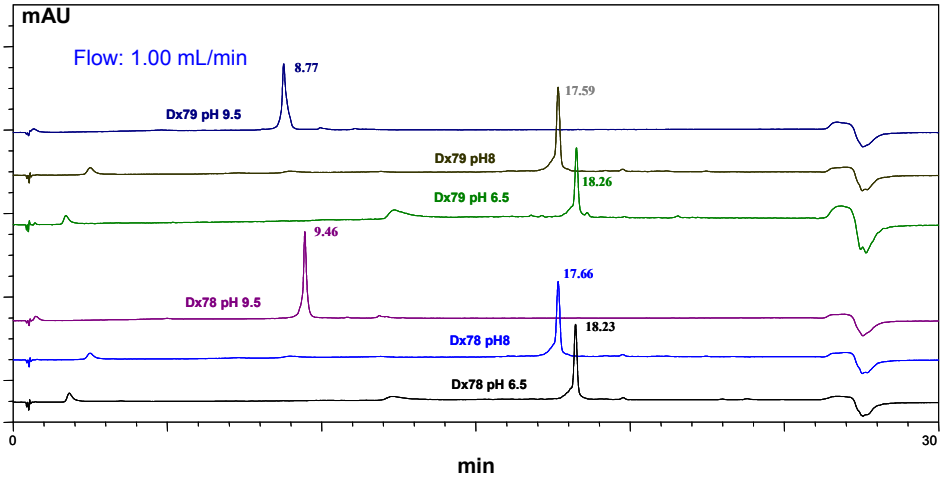


Figure 12
Effect of pH on Oligonucleotide Retention
ProSwift WAX, 78-825 mM NaCl in 25 min, 1 mL/min, 35 °C

4.3.3. Effect of Temperature on Oligonucleotide Retention

Here, a partially detritylated synthetic oligonucleotide was chromatographed at temperatures ranging from 10-60 °C using the same gradient. As the temperature increases, retention of both the tritylated and detritylated oligonucleotides increases; tritylated oligonucleotide retention increases to a greater degree than detritylated oligonucleotide retention. In this example, the tritylated oligonucleotide retention has increased to the point where it is not eluted, even at 1.125 M NaCl. Note that tritylated oligonucleotide peak shape improves as the temperature increases above 20 °C.

System:	Dionex ICS 2500
Column:	ProSwift WAX-1S
Flow:	1.0 mL/min
Gradient:	0.02M Tris to pH 8 with HCl
	300-1125 mM NaCl in 16 min
Detection:	Absorbance at 260 nm
Sample:	25 base partially detritylated oligonucleotide; 450 µg/mL.
Injection volume:	0.30 µL

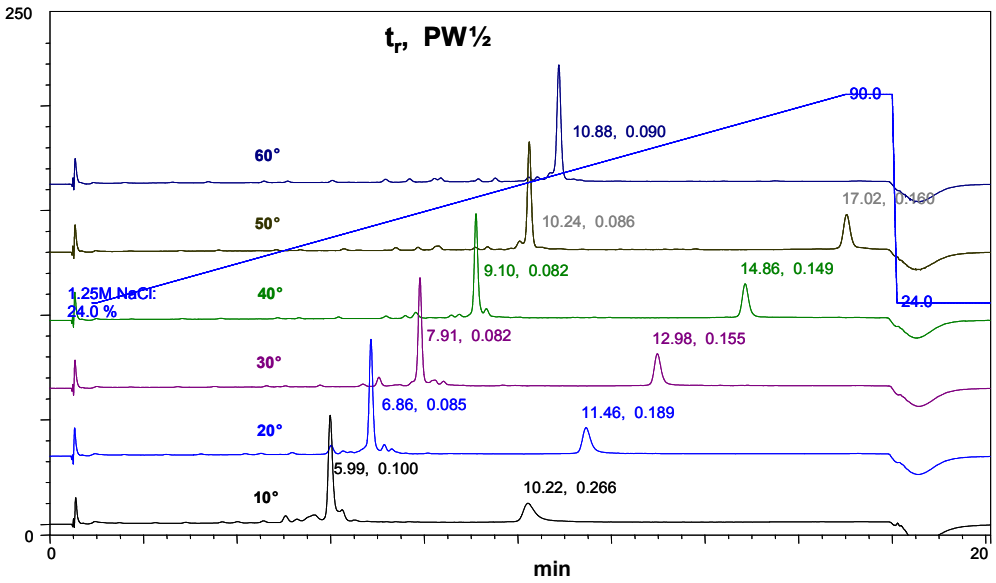


Figure 13
Effect of Temperature on ProSwift WAX ODN Elution
25 mer ± T; 300-1125 mM NaCl in 16 min, pH 8, 1.0 mL/min, 10-60 °C

4.3.4. Effect of Fluorophores and Capture Probes on Oligonucleotide Retention

Oligonucleotides harboring different fluorescent or affinity probes can be resolved from one another on ProSwift WAX columns (Figure 14). Thus, post-labeling purification of labeled oligonucleotides from their unlabeled parents is readily accomplished on the ProSwift WAX monolith.

System:	Dionex ICS 2500
Column:	ProSwift WAX-1S
Flow	1.0 mL/min
Gradient:	0.02M Tris to pH 8 with HCl 405-825 mM NaCl in 25 min
Detection:	Absorbance at 260 nm
Samples:	25 base oligonucleotides with: 1) no derivative 2) Biotin affinity ligand 3) Fluorescein 4) TET 5) TET and TAMRA (FRET labels)

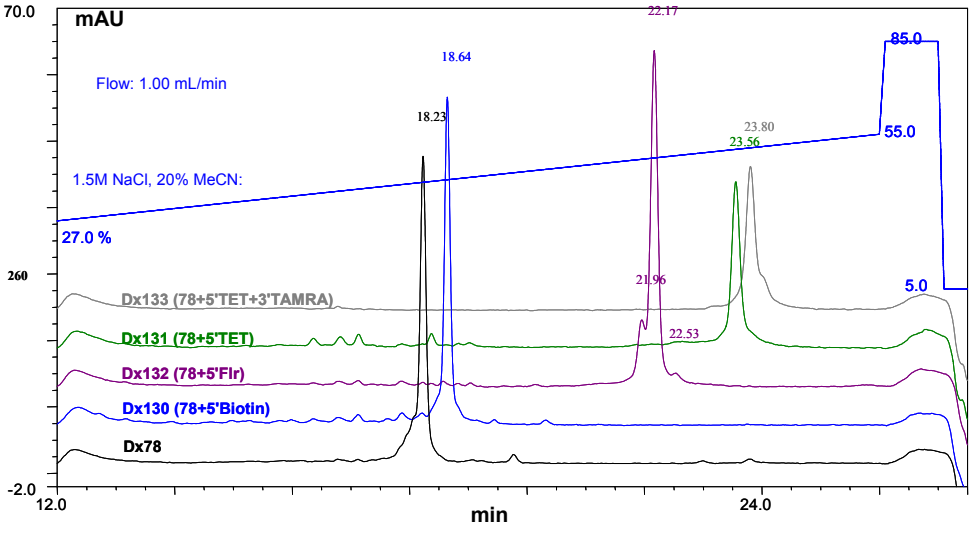


Figure 14
Effect of Derivative on WAX Monolith Retention

4.3.5. Effect of Oligonucleotide Loading Quantity on Peak Shape and Resolution

Synthetic oligonucleotides removed from the synthesizer before removal of the trityl protecting group are readily separated from their detritylated counterparts (Figure 15). This example shows also the effect of ON load on peak response and demonstrates that overloading the monolith still allows good separation of the full length ON from its detritylated failure sequences.

System:	Dionex ICS 2500
Column:	ProSwift WAX-1S
Flow	1.0 mL/min
Gradient:	0.02M Tris to pH 8 with HCl
	300-925 mM NaCl in 15 min
Detection:	Absorbance at 260 nm
	(0.4mm pathlength cell)
Samples:	Polymer mixed-base oligonucleotide in tritylated and Detritylated forms.

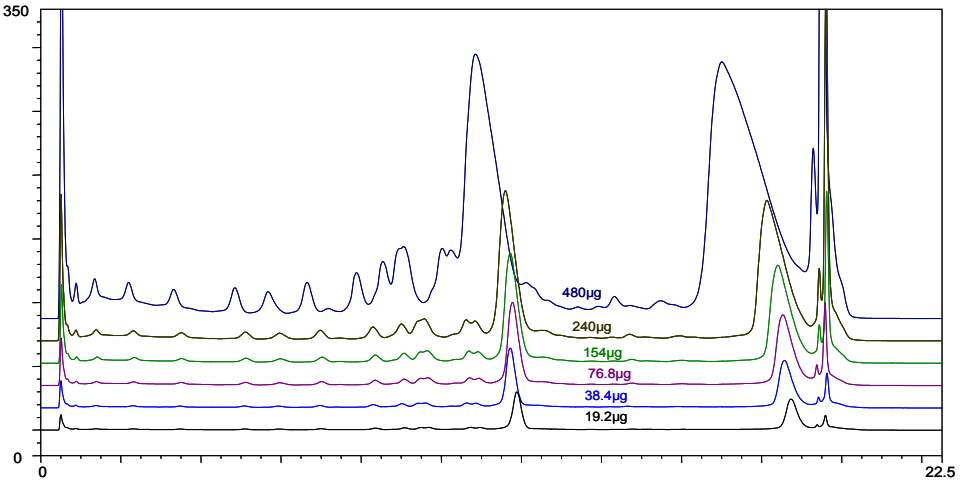


Figure 15
Loading Study of Swift WAX-1S 4.6 x 45mm Monolith
Ar25±T, pH 8, 45 C, 19-480 µg, PDA100 (with 0.4mm Cell)

Based on sample loading studies similar to that in the above chromatograms, Figure 16 shows the effect of oligonucleotide sample load on ProSwift Monolith peak width. The ProSwift Monolith shows good peak width up to ~ 120 µg per injection, and can be used for purification at significantly higher sample loads.

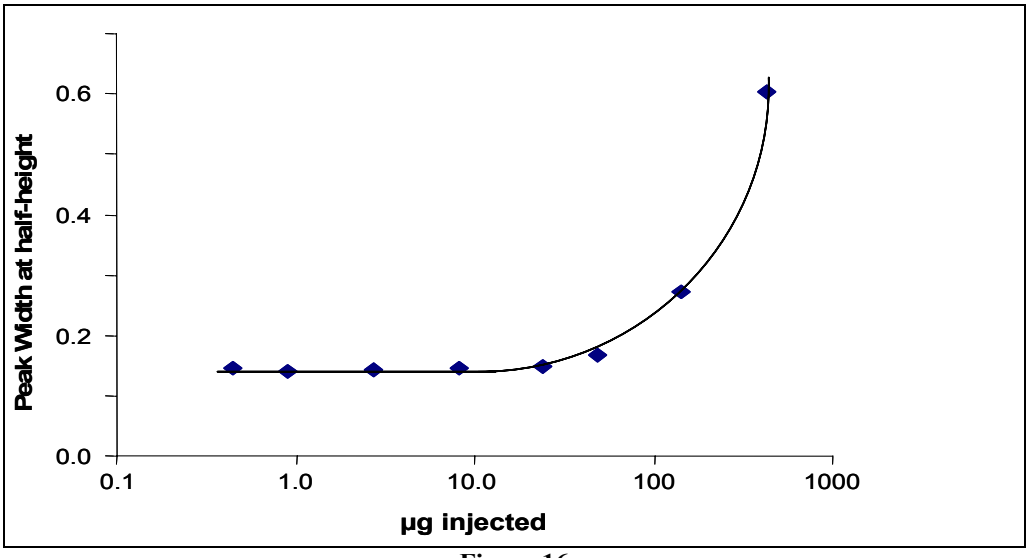


Figure 16
Dynamic Loading Capacity
ProSwift WAX 4.6 X 50 Monolith

5. TROUBLESHOOTING

The purpose of the Troubleshooting Guide is to help solve operating problems that may arise while using ProSwift WAX-1S columns. If you cannot solve the problem on your own, contact the Dionex North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390).

Table 4
WAX-1S Troubleshooting Summary

Observation	Cause	Action	Reference Section
High back pressure	Unknown	Isolate blocked component	5.1.1
	Plugged column bed supports	Clean, regenerate or replace column	5.1.2
	Other system modules	Disconnect, replace	System module manual
High background noise	Bad eluents	Remake eluents	5.2.1
	Contaminated column	Clean column	5.2.2
	Contaminated hardware	Clean component	5.2.3
Poor resolution	Poor efficiency due to large system void volumes	Re-plumb system	5.3.1.A
	Column headspace	Reverse column orientation.	5.3.1.B
	Contamination of column or frit	Clean column	5.3.2.C
Short retention times	Un-equilibrated system	Lengthen first eluent time before inject	5.3.2
	Flow rate too fast	Recalibrate pump or reduce flow	5.3.2.A
	Bad eluents	Remake eluents	5.3.2.B
	Column contamination	Clean column	5.3.2.C
Poor front end resolution	Bad eluents	Remake eluents	5.3.3.A
	Column overloading	Reduce sample size or concentration	5.3.3.B
	Insufficient column equilibration	Increase pre-equilibration time	5.3.3.C
	Sluggish injection valve	Service valve	5.3.3.D
	Large system void volumes	Re-plumb system	5.3.1.A, 5.3.3.E
Spurious peaks	Sample contamination	Pre-treat samples	5.4.A,
	Sluggish injection valve	Service valve	5.4.B
	Contaminated eluents	Test contamination, and remake eluents	5.4.C

5.1. High Back Pressure

5.1.1. Finding the Source of High System Pressure

If the system pressure is very high, it is advisable to find out what is causing the high system pressure.

The system should be used with a High-Pressure In-Line Eluent Filter. The filter should be positioned between the pump and the injection valve. Make sure you have a High-Pressure In-Line Filter in place and that it is not contaminated.

A. Make sure that the pump is set to the correct eluent flow rate. Higher than recommended eluent flow rates will cause higher pressure. Measure the pump flow rate at various flow rates, if necessary by collecting the flow of deionized water into a pre-weighed graduated cylinder. Calculate the flow rate based on the collected volume of deionized water.

B. Find out what part of the system is causing the high pressure. It could be a piece of tubing that has plugged or whose walls have collapsed, an injection valve with a plugged port, a column with particulates plugging the bed support, a plugged High-Pressure In-Line Filter, or the detector cell.

To find out which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure. It should not exceed 50 psi (0.34 MPa). Continue adding the system components (injection valve, column(s), and detector) one by one, while watching the system pressure. The pressure should increase up to a maximum of 600 psi (4.2 MPa) at a flow rate of 1.0 mL/min when the WAX-1S column is connected. No other components should add more than 50 psi (0.34 MPa) of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

5.1.2. Clogged Column Bed Support Assemblies

If the column inlet frit or the media is determined to be the cause of the high back pressure, clean the column in the reversed direction, or regenerate the columns using the methods described in Appendix B.2.2. Replace the column if the cleaning and regeneration does not help.

5.2. High Background or Noise

5.2.1. Contamination of Eluents

A. Make sure that all eluents are made correctly, and from chemicals with the recommended purity.
B. Make sure that the deionized water used to prepare the reagents has a specific resistance of 18.2 megohm-cm.

5.2.2. Contaminated Column

Remove the ProSwift WAX-1S column from the system. If the background noise decreases, then the column itself is the cause of the high background. Clean the column as instructed in Appendix B - Column Care.

5.2.3. Contaminated Hardware

To eliminate the hardware as the source of the high background signal, bypass the column and pump deionized water with a specific resistance of 18.2 megohm-cm through the system. The background signal should be less than 0.1 mA_{260}. If it is not, check the detector cell by injecting deionized water directly into it. See the appropriate manual for further details.

5.3. Poor Peak Resolution

Poor peak resolution can be due any or all of the following factors.

5.3.1. Loss of Column Efficiency

a. Ensure that system void volumes have been minimized. Extra-column volumes can cause sample band dispersion and will decrease peak efficiencies. Make sure you are using tubing with an I.D. $\leq$ 0.010" to make all eluent liquid line connections between the injection valve and the detector cell inlet on 4-mm systems. Make all tubing lengths as short as possible. Check for leaks.

b. Contamination of media or frit due to binding of sample or eluent components. This can be responsible for the loss of column efficiency. Please refer to Appendix B.2.2- Column cleanup procedure.

5.3.2. Poor Resolution Due to Shortened Retention Times

Even with adequate system and column efficiency, resolution of peaks will be compromised if the analytes elute too early.

A. Check the eluent flow rate. If it is different than the flow rate specified by the analytical protocol, recalibrate the pump. Measure the eluent flow rate as described in section 5.1.1.A.

B. Check to ensure that the eluent compositions and concentrations are correct.

For isocratic analysis, an eluent that is too strong will cause the peaks to elute too early. Prepare fresh eluent. If you are using a gradient pump to proportion the final eluent from concentrated eluents in two or three different eluent reservoirs, the composition of the final eluent may not be accurate enough for the application. Use one reservoir containing the correct eluent composition to see if this is the problem. This may be a problem when one of the proportioned eluents is less than 5%.

For gradient analysis, remake the eluents or adjust the times in the gradient program to obtain the required peak resolutions.

C. Column contamination can lead to a loss of column capacity because fewer of the binding sites will be available for the sample compounds. Polymers or metal ions might be concentrating on the column. Refer to Appendix B.2.2 - Column Cleanup Procedure, for column cleanup recommendations.

Possible sources of column contamination are impurities in chemicals or components in the sample matrix. Be especially careful to make sure that the recommended chemicals are used. The deionized water should have a specific resistance of at least 18.2 megohm-cm, and the solvents should be of HPLC-grade.

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column. If you need assistance in solving resolution problems, contact the nearest Dionex Office.

5.3.3. Loss of Front End Resolution

If poor resolution and efficiency is observed for very early eluting peaks (near the system void volume) compared to the later eluting peaks, check the following:

A. Improper eluent concentration may be the problem. Remake the eluent as required for your application. Ensure that the water and chemicals used are of the required purity.

B. Column overloading may be the problem. Reduce the amount of sample injected onto the column by either diluting the sample or injecting a smaller volume onto the column.

C. The column may not be equilibrated with the first eluent. Increase the amount of time the first eluent runs through the columns before injection.

D. Sluggish operation of the injection valve may be the problem. Check the valve operation to make sure there are no leaks or partially plugged port faces. Refer to the valve manual for instructions.

E. Improperly swept out volumes anywhere in the system prior to the columns may be the problem. Swap components (one at a time) in the system prior to the analytical column and test for front-end resolution after every system change.

5.4. Spurious Peaks

A. The column may be contaminated. If the samples contain an appreciable level of ionic components and the column is used with a weak eluent system, these components may remain on the analytical column. The retention times for the analytes in subsequent injections will then decrease, and spurious, inefficient (broad) peaks can show up at unexpected times. Clean the column as indicated in "Appendix B.2.2 - Column Cleanup Procedure."

B. The injection valve may be creating a baseline disturbance. This baseline upset can show up as a peak of varying size and shape. It will happen when the injection valve needs to be cleaned or torque (see valve manual). Check to see there are no restrictions in the tubing connected to the valve. Also check the valve port faces for blockage and replace them if necessary. Refer to the Valve Manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked as long as they do not interfere with the quantification of the peaks of interest. If cleaning and torque the valve does not help, replace the valve.

C. The eluent may be contaminated. When performing gradient chromatography, contaminants in the eluent may accumulate on the column until the eluent strength is sufficient to elute them. Increasing the equilibration time at low eluent strength will result in more pronounced contaminant peaks. If this is observed, make the eluents again. If the problem persists, prepare the eluents from higher purity chemicals.

D. If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the ProSwift WAX-1S columns, please contact Dionex Technical help at 1-800-Dionex-0 (1-800-346-6390)

5.5. Small Peak Areas

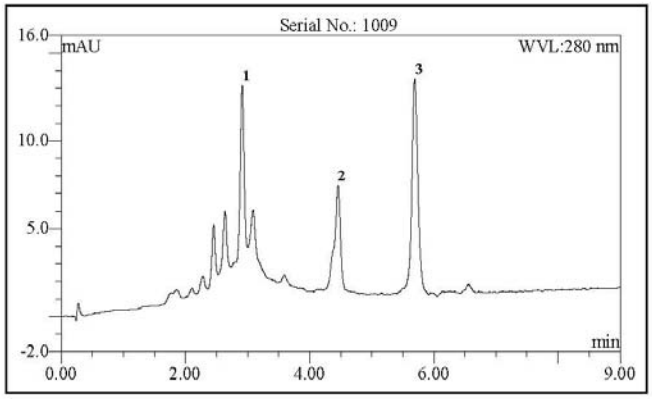
Small peak areas can occur when the injection valve is incorrectly installed or controlled. Plumb the injection valve's sample loop so that it is inserted into the flow path when the inject command is issued.

APPENDIX A - QUALITY ASSURANCE REPORT

ProSwift™ WAX-1S
4.6 x 50 mm
Product No. 064294

Date: 30-Mar-06 22:18
Serial No. : 001009
Lot No. : 006-06-012

Eluent A: 10 mM Tris, pH 7.6
Eluent B: 1M Sodium Chloride in 10 mM Tris, pH 7.6
Gradient: 0% to 75% B in 9 minutes
Flow Rate: 2.0 mL/min
Temperature: Ambient
Detection: UV 280 nm
Injection Volume: 10 µL
Storage Solution: 0.05M NaCl in 10 mM Tris, pH 7.6 + 0.1% Sodium Azide



No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Peak Width (50%) (min)	Concentration (mg/mL)
1	Ovalbumin	2.91	0.6	11.01	0.077	1.0
2	Trypsin Inhibitor	4.46	0.5	7.73	0.089	1.0
3	Insulin Chain A	5.69	1.2	n.a.	0.099	0.25

QA Results:

Component	Parameter	Specification	Results
	Pressure	<=660	435
Insulin Chain A	Asymmetry	1.0-1.5	Passed
Insulin Chain A	PW (50%)	0.070-0.113	Passed
Insulin Chain A	Ret. Time	5.59-5.87	Passed
T1/T2	Ret.Time Ratio	0.71-0.91	Passed

T1 = Ret.Time of Insulin Chain A minus Trypsin Inhibitor
T2 = Ret.Time of Trypsin Inhibitor minus Ovalbumin

Production Reference:
Datasource: MonoBio 6.70 SP2a Build 1871

Chromeleon® Dionex Corp. 1994-2006

APPENDIX B - COLUMN CARE

B.1 New Column Equilibration

The columns are shipped in the storage solution containing 0.1% NaN_3 to suppress microbial growth. Before use, please refer to QuickStart (Section 3.1) for cleaning of the storage solution.

B.2 Column Cleanup

If the column inlet frit or the media is fouled by sample or eluent contaminants, protein precipitates and hydrophobic bounding proteins, these may be removed by a strong solvent, solubilizing agents, acid, base, or pepsin treatment.

CAUTION

Always ensure that the cleanup protocol used does not switch directly between eluents that can react or precipitate when mixed together. Use pure Also chose the flow rate which will not create higher column backpressure than maximum pressure in column specifications.

B.2.1 Cleanup Solution

Contaminants	Clean up solutions	Amount
General contaminants	2 M NaCl 1 M NaOH	4 CV (3 mL) 4 CV (3 mL)
Hydrophobic bounding proteins	≤ 100% CH_3CN ≤ 2 M NaOH ≤ 75% Acetic acid ≤ 100% Ethanol ≤ 0.5 % non-ionic detergents (in 0.1 M acetic acid solution)	4 CV (3 mL) 4 CV (3 mL) 4 CV (3 mL) 4 CV (3 mL) 4 CV (3 mL) 4 CV (3 mL)
Protein Precipitates	6 M guanidine hydrochloride	4 CV (3 mL)

B.2.2 Column Cleanup Procedure

1. Preparation:
   a. Eluent Preparation
      Eluent A: 0.01 M Tris.HCl buffer, pH 7.6
      Eluent B: 2 M NaCl in 0.01 M Tris.HCl buffer pH 7.6

   b. Column Installation
      Install the column on the LC instrument in the reversed flow direction and a 1-3 mL injection loop.

   c. Flow Rate Set Up:
      Set the pump flow rate to ≤0.50 mL/min.

WARNING

If your eluent composition generates back pressure in excess of the maximum operation pressure, adjust the flow rate to ensure the backpressure of the column is less than the recommended maximum operation pressure.

2. Column Cleaning Procedures:
   a. Run a LC method using the following program:
      - Pump 4 CV (ca. 3 mL for 4.6 x 50 mm column) 100% eluent A.
      - Pump 20 CV (15 mL) gradient from 100% A to 100% eluent B.
      - Pump 4 CV (3 mL) of 100% eluent B.
      - Pump 4 CV (3 mL) reversed gradient from 100% B to 5% eluent B.
      - Pump 4 CV (3 mL) of 5% eluent B.

b. Load and Inject Cleaning Solutions:
   - Maintain the flow of 5% eluent B.
   - Load and inject a cleaning solution of your choice (see list of solvents in the above table).
   - Pump 4 CV (3 mL) of 5% eluent B.
   - Continue to load and inject other cleaning solutions of your choices.
   - Pump 4 CV (3 mL) of 5% eluent B.

c. Repeat the above cleaning steps with the same or different cleaning solvents until a constant baseline is achieved during the gradient run.

3. Column Re-equilibration:
   a. Reconnect the ProSwift column in its proper orientation.

   b. Equilibrate the column with eluent before resuming normal operation.

B.3 Column Storage

B.3.1 Short Term Storage:

For short term storage (less than 3 days), use the low salt concentration eluent (pH = 3 - 10) as the column storage solution.

B.3.2 Long Term Storage:

For long term storage, use the following solution to avoid microbial growth on the column.

- 0.05 M NaCl
- 0.1% NaN_3
- 0.01 M Tris.HCl at pH 7.6

Flush the column with at least 10 mL of the storage eluent. Cap both ends securely using the plugs supplied with the column.

APPENDIX C - REFERENCES

1. High Speed Separation of Oligonucleotides using SWIFT WAX monolithic column, B. Kunnummal, S. Xie, T. Jiang, L. Xu and R. W. Allington, *PharmaGenomics*, 8, March/April 2004.
2. Porous Polymer Monoliths: An Alternative to Classical Beads. S. Xie, R. W. Allington, F. Svec, J. M. J. Fréchet, Advances in Biochemical Engineering/Biotechnology Spec. Vol: Modern Advances in Chromatography, Ed. Dr. R. Freitag, 2002.