
# ProPac WAX-10 / SAX-10 Columns

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Thermo SCIENTIFIC

*For Research Use Only. Not for use in diagnostic procedures*

# Product Manual

# for

## ProPac WAX-10G Guard Column

4 × 50 mm, P/N 055150
2 × 50 mm, P/N 063470

## ProPac WAX-10 Analytical Column

22 × 250 mm, P/N 088771
9 × 250 mm, P/N 063707
4 × 250 mm, P/N 054999
2 × 250 mm, P/N 063464

## ProPac SAX-10G Guard Column

4 × 50 mm, P/N 054998
2 × 50 mm, P/N 063454

## ProPac SAX-10 Analytical Column

22 × 250mm, P/N 088770
9 × 250mm, P/N 063703
4 × 250 mm, P/N 054997
4 × 50mm, P/N 078990
2 × 250mm, P/N 063448

Revision History:

Revision 05, August 6, 2012, Reformatted for Thermo Scientific. Added SAX-10 Analytical Column (4 x 50mm, P/N 074600).

Revision 06, October, 2015, Added new part numbers.

## Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

| | |
|---|---|
|  **SAFETY** | ***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.*** |
|  **WARNING** | ***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.*** |
|  **CAUTION** | ***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.*** |
|  **NOTE** | ***Indicates information of general interest.*** |
| **IMPORTANT** | ***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.*** |
| **Tip** | ***Highlights helpful information that can make a task easier.*** |

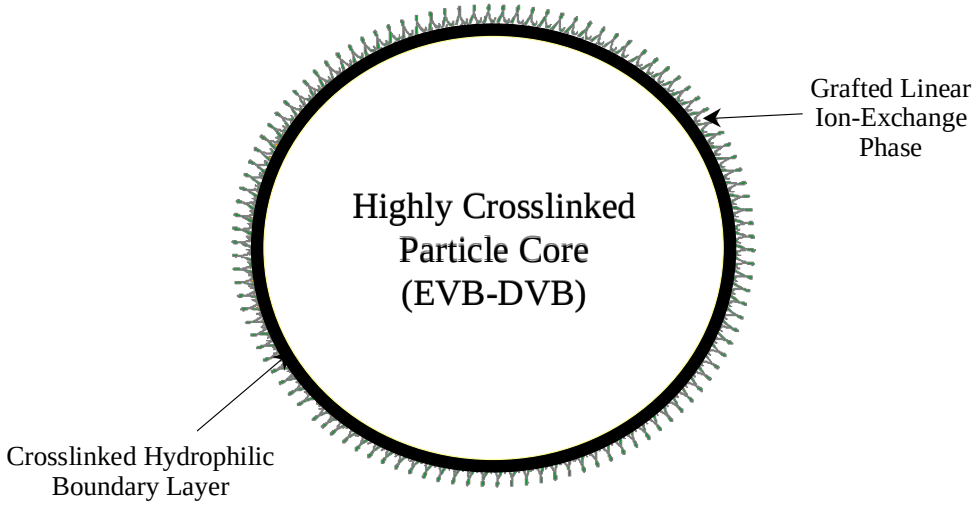
# Contents

# 1. Introduction

The Thermo Scientific ProPac protein columns are specifically designed to provide high-resolution and high efficiency separations of proteins and glycoproteins pI = 3 - 10 MW: > 10,000 units.

The packing material is composed of a 10 µm, solvent compatible, microporous ethylvinylbenzene cross-linked with 55% divinylbenzene polymer substrate. This resin is covered with a highly hydrophilic, neutral polymer, to minimize non-specific interactions between the surface and the biopolymer. On the hydrophilic layer a controlled polymer chain is grafted to introduce the anion exchange functionality. For the weak anion exchange column (ProPac™ WAX-10), the surface is grafted with a polymer chain bearing tertiary amine groups. For the strong anion exchanger (ProPac SAX-10), the surface is grafted with a polymer chain bearing quarternary ammonium groups. Figure 1 below illustrates this surface structure.

**Figure 1 Schematic Diagram of the ProPac Phase for Protein Separations**



# 2. Installation

The ProPac columns were designed to be used with a standard bore HPLC system having a gradient pump module, injection valve and a UV-Vis detector.

A metal-free pump system is recommended for halide-salt eluents which may cause corrosion of metallic components leading to decreased column performance from metal contamination. A metal- free pump is recommended to avoid denaturation of the protein samples. Use of stainless steel tubing, ferrule and bolt assemblies is not recommended because they may damage the threads of the PEEK end fittings.

## 2.1 System Void Volume

Tubing between the injection valve and detector should be ≤ 0.010" I.D. PEEK tubing. Minimize the length of all liquid lines, but 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 for small ID columns.

## 2.2 Operational Parameters

| | |
|---|---|
| pH Range: | pH = 2 - 12 |
| Temperature limit: | 60°C |
| Pressure limit: | 3,000 psi |
| Organic Solvent Limit: | 80% acetonitrile or acetone if required for cleaning. |
| Detergent compatibility: | Nonionic, cationic or zwitterionic detergents.<br>**Do not use anionic detergents.** |
| Typical eluents: | Sodium, potassium salts of phosphate, chloride, or acetate. |

| | |
|---|---|
| Flow rate (recommended): | 2 x 250mm: 0.25 mL<br>4 x 150mm & 4 x 100mm: 1.0 mL/min<br>4 x 250mm: 1.0 mL/min<br>9 x 250mm: 5 mL/min*<br>22 x 250mm: Upto 30 mL/min* |

(*Maximum pressure 3000 Psi)

| | |
|---|---|
| Column Volume | 4 x 250 mm = 3.14 mL<br>9 x 250 mm = 15.7 mL<br>22 x 250 mm = 94.2 mL |
| Capacity* | ProPac WAX-10 = 5 mg/mL BSA<br>ProPac SAX-10 = 15 mg/mL BSA |

*Breakthrough Capacity: Depending on the protein, 10 – 100 µg protein can be injected

| | |
|---|---|
| Dynamic capacity<br>(Suggested Loading amount) | 2 x 250mm: 25 µg<br>4 x 50 mm: 20 µg<br>4 x 100mm: 40 µg<br>4 x 150mm: 60 µg<br>4 x 250mm: 100 µg<br>9 x 250mm: 500 µg<br>22 x 250mm: 3000 µg |

### 2.2.2 Physical Characteristics

| FEATURE | SPECIFICATION |
|---|---|
| Substrate Particle Size | 10 µm |
| Substrate Pore Size | Non-porous |
| Substrate Monomers | ethylvinylbenzene-divinylbenzene |
| Substrate Cross-linking | 55% |
| Mode of Interaction | Anion Exchange |
| Functional Group | WAX-10 - Tertiary amine<br>SAX-10 - Quaternary ammonium |

## 2.3 Eluent Limitations

The ProPac 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 is usually unnecessary. If you decide to use one, test the solubility limit of eluents in the presence of the chosen organic solvents. Some combinations of eluent salts and organic solvents are not miscible.

**NOTE**

***Anionic detergents will irreversibly bind to the column and their use should be avoided.***

## 2.4 Chemical Purity Requirements

Obtaining reliable, 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 has been compromised.

### 2.4.1 Inorganic, Organic Chemicals

Reagent grade or better inorganic chemicals should always be used to prepare eluents. Whenever possible, inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity should be used. These chemicals will detail the purity by having an actual lot analysis on each label.

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

### 2.4.2 Deionized Water

The deionized water used to prepare eluents should be Type I Reagent Grade Water with specific resistance of 18.2 megohm-cm. The deionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm.

## 2.5 Eluent Preparation

### 2.5.1 Adjusting the pH of the Eluent

The eluent solution should contain all the electrolytes before adjusting the pH. To make sure that the pH reading is correct, the pH meter needs to be calibrated at least once a day. Stirring and temperature correction should be employed. Care should be taken to ensure the accuracy of the pH electrode for Tris buffers. Some electrodes will give erroneous results with Tris.

### 2.5.2 Filtering the Eluent

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

### 2.5.3 Degassing the Eluent

Before use, the eluents must be degassed. The degassing can be done using a vacuum pump. Vacuum degas the solvent by placing the eluent reservoir in a sonicator and drawing vacuum on the filled reservoir with a vacuum pump for 5-10 minutes while sonicating.

# 3. Operation

## 3.1 Sample Preparation

The protein samples are best dissolved in the initial run buffer or in pure D.I. water. The concentration should be determined so the column is not overloaded by the injected sample. The loading capacity of the column is about 10 - 100 µg protein/column; the sample loop typically used for the 4 x 250 mm column size is 10 - 100 µL. If the protein sample contains particulate contamination, the sample should be filtered through a 0.2 µm syringe filter.

## 3.2 Column Equilibration

The WAX-10 is shipped in 20 mM Tris pH 8.0/0.1% sodium azide.

The SAX-10 is shipped in 10 mM Tris pH 8.5/0.1% sodium azide.

Before performing a run, equilibrate the column with the starting run buffer using approximately 10 times the column volume (i.e. 15 mL in the case of a 4 x 250 mm column). After cleaning the column or when switching to a different buffer type, a longer equilibration time is recommended. Use an eluent volume of 10 times the column volume to ensure the column is well equilibrated.

## 3.3 Test Chromatograms

### 3.3.1 Production Test Chromatogram - SAX-10

Each column is individually tested to ensure the quality of the product. A tight set of tolerances surround the final test chromatogram to ensure low column to column variability for the protein applications the columns will undertake. Examples of the test chromatograms are shown below.

**Eluent:**

E1: 10 mM Tris pH = 8.50
E2: 10 mM Tris + 0.5 M NaCl pH = 8.50

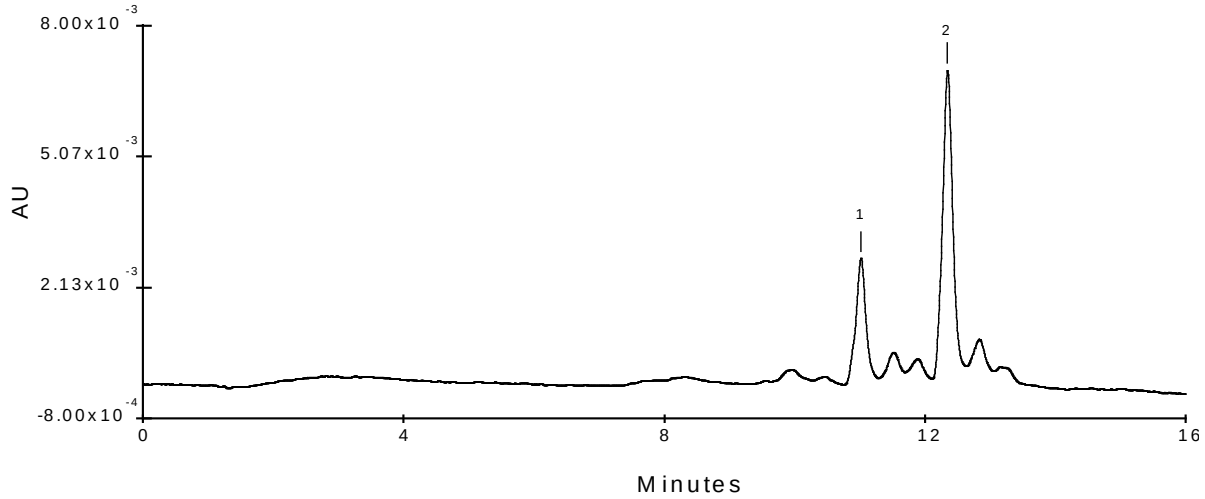
**Gradient:**

| Time | % E1 | % E2 | Comment |
|---|---|---|---|
| 0.0 | 100 | 0 | |
| 0.4 | 100 | 0 | Inject Sample |
| 0.5 | 100 | 0 | Start Data Collection |
| 15.0 | 50 | 50 | |
| 16.0 | 0 | 100 | |
| 17.0 | 100 | 0 | |
| 25.0 | 100 | 0 | |

**Flow Rate:** 1.0 mL/min
**Detection:** UV at 280 nm
**Storage Solution:** E1 + 0.1% sodium azide
**Injection Volume:** 10 µL

**Analytes:**
1. Ovalbumin 1
2. Ovalbumin 2

**Figure 2 ProPac SAX-10 (4 x 250 mm) Test Chromatogram**



### 3.3.2 Production Test Chromatogram – ProPac WAX-10

Each column is individually tested to ensure the quality of the product. A tight set of tolerances surround the final test chromatogram to ensure low column to column variability for the protein applications the columns will undertake. Examples of the test chromatograms are shown below.

**Eluent:**

| | |
|---|---|
| E1: | 20 mM Tris pH = 8.00 |
| E2: | 20 mM Tris + 0.5 M NaCl pH = 8.00 |

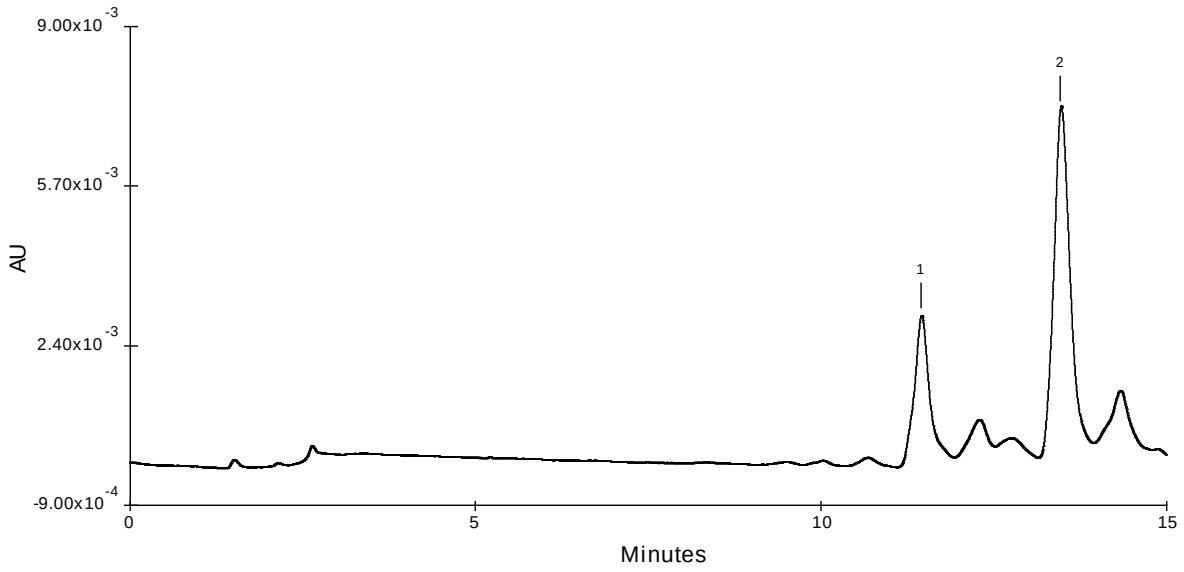
**Gradient:**

| Time | % E1 | % E2 | Comment |
|---|---|---|---|
| 0.0 | 100 | 0 | |
| 0.4 | 100 | 0 | Inject Sample |
| 0.5 | 100 | 0 | Start Data Collection |
| 15.0 | 50 | 50 | |
| 15.1 | 0 | 100 | |
| 17.0 | 0 | 100 | |
| 17.1 | 100 | 0 | |
| 25.0 | 100 | 0 | |

**Flow Rate:** 1.0 mL/min
**Detection:** UV at 280 nm
**Storage Solution:** E1 + 0.1% sodium azide
**Injection Volume:** 10 µL

**Analytes:**
1. Ovalbumin 1
2. Ovalbumin 2

**Figure 3 ProPac WAX-10 (4 x 250 mm) Test Chromatogram**



# 4. Example Applications

## 4.1 Elution Profiles on a ProPac SAX-10 Anion-Exchange Column

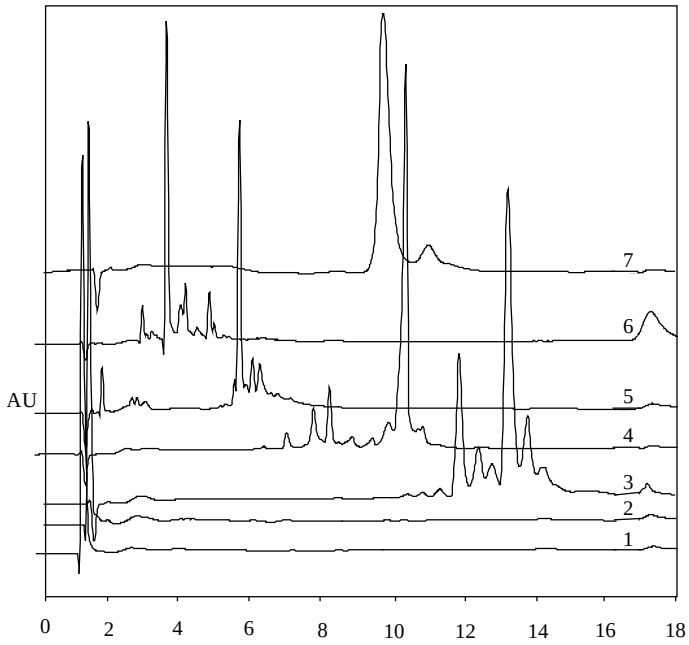
A series of proteins were chromatographed to give a general impression of the capability of the ProPac anion exchange column. Elution profiles for a couple of basic proteins, lysozyme and cytochrome c, are shown to demonstrate that the surface of the column possesses only an anion exchange characteristic and that residual cation exchange sites are absent, as evidenced by the lack of retention for basic proteins. Tryspin inhibitor is also shown as it has been reported that it is not always possible to resolve all three inhibitors in anion exchange. Ovalbumin has been noted to have two possible phosphorylation sites could result in a series of closely related variants. In the literature it has been shown that creatine kinase has four closely related forms which have pI values which differ by about 0.1 pH unit. Elution profiles for transferrin are shown to demonstrate the selectivity the column demonstrates towards variations in protein sialyation. BSA is also known to exist in solution with a small percentage in the dimerized form.

| | |
|---|---|
| Column: | ProPac SAX-10, 4 x 250 mm |
| Eluent: | A) Water |
| | B) Water |
| | C) 2.0 M NaCl |
| | D) 0.2 M Tris/HCl, pH 8.5 |
| Gradient: | A) 0 - 0.5 M NaCl in 15 min |
| | B) 0 - 0.25 M NaCl in 15 min |
| | C) 0 - 0.25 M NaCl in 30 min. |
| | 20 mM Tris/HCl throughout |
| Flow Rate: | 1.0 mL/min |
| Injection Amount | 50 µL (1 mg/mL) |
| Detection: | 214 nm |

**Samples:**

1. Lysozyme (B)
2. Cytochrome c, bovine (B)
3. Ovalbumin (B)
4. Trypsin inhibitor, soy (A)
5. Creatine kinase, rabbit (B)
6. Carbonic anhydrase (A)
7. BSA (A)

**Figure 4 Elution Profiles on a ProPac SAX-10 Strong Anion-Exchange Column**



## 4.2 Effect of Acetonitrile and Temperature on the Elution Profiles of Ovalbumin

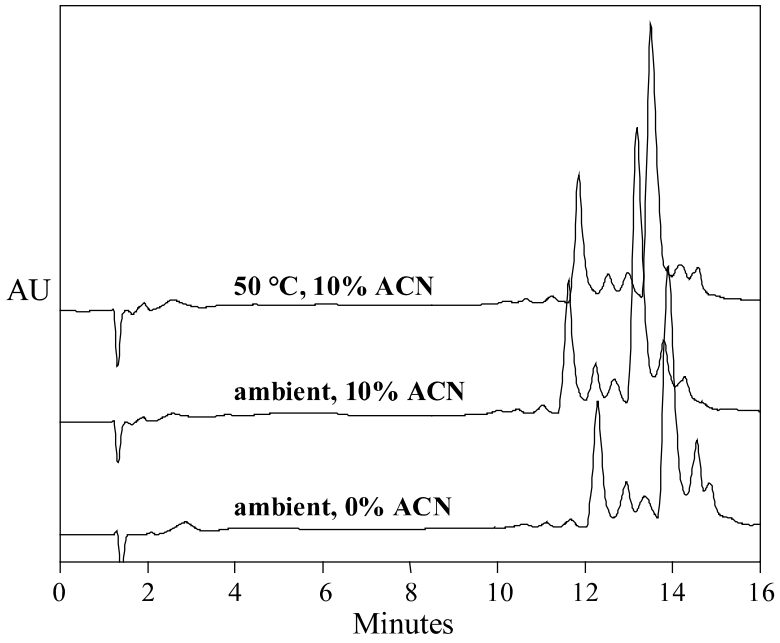
In this evaluation it was demonstrated that the column exhibited minimal kinetic resistances and that no appreciable secondary hydrophobic interactions were observed. This result was obtained for a series of proteins including trypsin inhibitor, carbonic anhydrase, transferrin, creatine kinase and ovalbumin for which data is presented.

By increasing the temperature at which the chromatography is conducted the rates associated with diffusion and the kinetics of binding are increased. As no significant change is observed in the elution profiles as function of temperature it can be inferred that such effects do not significantly affect the performance of the column at room temperature. Likewise, for hydrophobic interactions the similarity of the elution profiles of the proteins with and without acetonitrile, which will reduce any hydrophobic interaction between the protein and the stationary phase, implies that hydrophobic interactions are essentially absent.

**Column:** ProPac SAX-10, 4 x 250 mm
**Eluents:** A) Water
B) Water, 20% v/v ACN
C) 2.0 M NaCl
D) 0.2 M Tris/HCl (pH 8.5)
**Gradient:** 20 mM Tris/HCl 0 - 25 min
0 - 0.50 M NaCl; 0 - 15 min
0.5 M NaCl; 15 - 17 min
0 M NaCl; 17 - 25 min
**Flow Rates:** 1.0 mL/min
**Inj. Amt:** 50 µg (1mg/mL)
**Detection:** 214 nm
**Samples:** Ovalbumin

**Samples:**
1. Lysozyme (B)
2. Cytochrome c, bovine (B)
3. Ovalbumin (B)
4. Trypsin inhibitor, soy (A)
5. Creatine kinase, rabbit (B)
6. Carbonic anhydrase (A)
7. BSA (A)

**Figure 5 Effect of Acetonitrile and Temperature on the Elution Profiles of Ovalbumin**

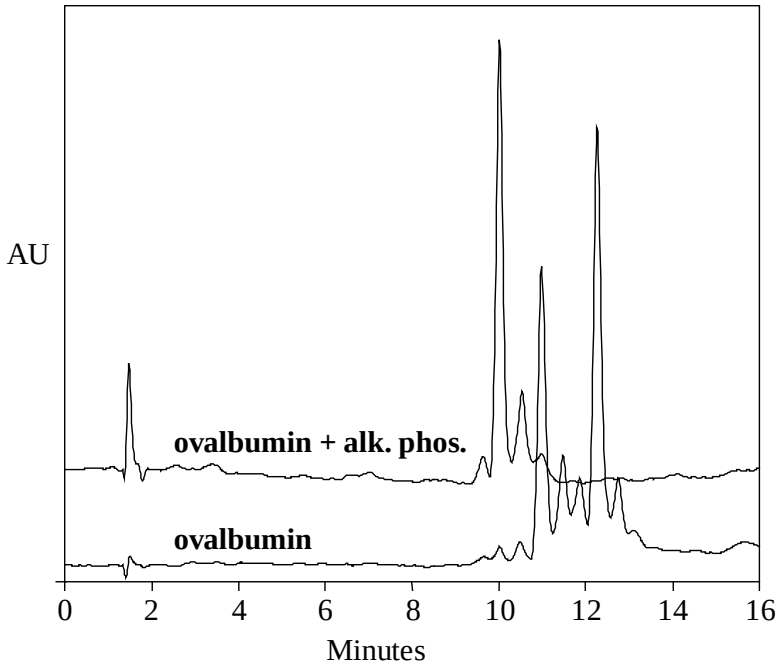


## 4.3 Effect of Alkaline Phosphatase on Ovalbumin Elution Profiles on an Anion-Exchange Analytical Column

Resolution of phosphorylation variants is important in the characterization of bio-macromolecules, see *e.g.* [4] and references within. We resolved several phosphorylation isoforms of ovalbumin using a simple linear gradient on the ProPac Strong Anion Exchange column. It is seen that eight peaks are visible in the ovalbumin chromatogram profile. Upon alkaline phosphatase digestion of ovalbumin to remove phosphate from the protein, the ovalbumin profile simplifies from eight peaks to one major and three minor peaks. The modification(s) responsible for the three minor peaks has not been identified.

| | |
|---|---|
| **Columns:** | ProPac SAX-10, 4 x 250 mm |
| **Eluents:** | A) Water<br>B) Water<br>C) 2.0 M NaCl<br>D) 0.2 M Tris/HCl (pH 8.5) |
| **Gradient:** | 20 mM Tris/HCl; 0 - 25 min<br>0.0 - 0.25 M NaCl; 0 - 15 min<br>0.5 M NaCl; 17 - 19 min<br>0.0 M NaCl; 17 - 25 min |
| **Flow Rates:** | 1.0 mL/min |
| **Inj. Amt:** | 30 µg (1 mg/mL) |
| **Detection:** | 214 nm |
| **Samples:** | Ovalbumin before and after treatment with alkaline phosphatase treatment |

**Figure 6 Effect of Alkaline Phosphatase on Ovalbumin Elution Profiles on a Strong Anion-Exchange Analytical Column**

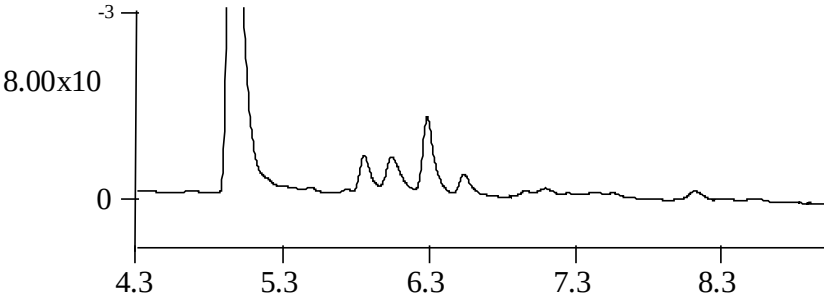


## 4.4 Selectivity Comparison of Anion-Exchange Columns

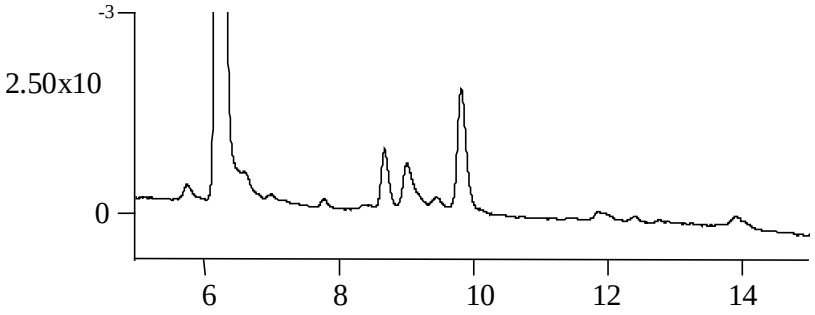
ProPac SAX-10 and ProPac WAX-10 have high selectivity for proteins. These columns can even separate the proteins with minor components, one charge difference and minor structure variations. One example shown here is the separation of carbonic anhydrase from the minor components.

**Figure 7 High Selectivity of Anion-Exchange Column**

**Columns:** ProPac SAX-10, 4 x 250 mm
**Eluents:** 10 mM Tris (pH 8.5)
0.0 - 0.15 M NaCl; 0 - 15 min
**Flow Rates:** 1.0 mL/min
**Inj. Amt:** 10 µL
**Detection:** 214 nm
**Samples:** Carbonic anhydrase




**Columns:** ProPac WAX-10, 4 x 250 mm
**Eluents:** 10 mM Tris (pH 8.0)
0.0 - 0.1 M NaCl; 0 - 30 min
**Flow Rates:** 1.0 mL/min
**Inj. Amt:** 10 µL
**Detection:** 214 nm
**Samples:** Carbonic anhydrase



## 4.5 Effect of Sialytion on Transferrin Chromatography

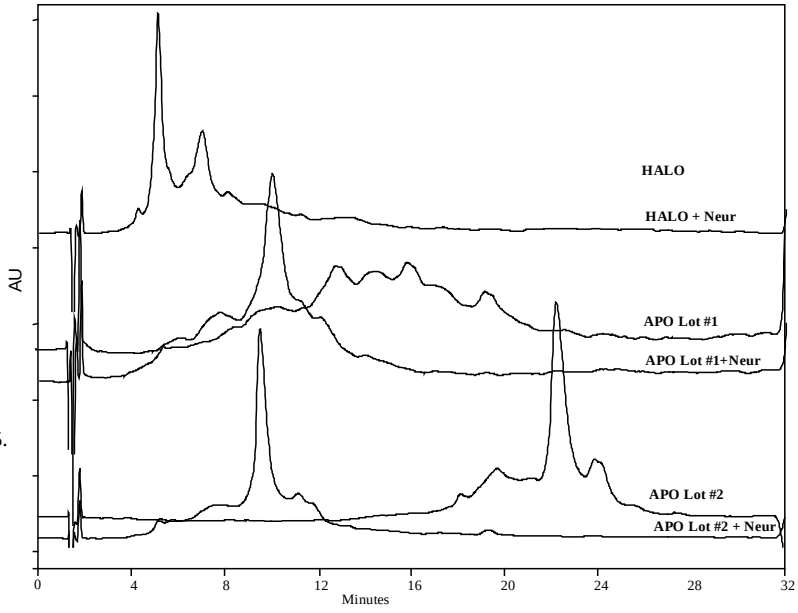
Transferrins are a group of metal-binding glycoproteins, which function in the transport of iron in cells. Human transferrin has two iron binding sites and has a molecular mass of ~ 75,000 daltons. It has two N-linked glycosylation sites ($Asn^{413}$ and $Asn^{611}$) which are occupied by bi-, tri- or tetra-antennary N-acetyllactosamine oligosaccharides [1].

Recent data suggests that different isoform profiles of transferrin are diagnostic of different clinical conditions and may be clinically significant. For example it is known that pregnant women in their last trimester have transferrin with increased oligosaccharide branching and increased sialylation. Alternatively, alcoholics exhibit decreased sialylation of transferrin, an alteration in their isoform profile, which is reversible with abstinence [2].

In this application we demonstrate that elution profiles of different transferrins result from differences in the sialylation of the protein, see [3]. Three transferrin samples, one iron rich (Holo) and two from different iron poor (Apo) manufacturers lots, exhibited unique isoform profiles by anion exchange on the ProPac column. When the different transferrin samples are digested with neuraminidase to remove sialic acid, the profiles collapse into a similar pattern.

### Figure 8 Effect of Sialytion on Transferrin Chromatography

**Columns:** ProPac SAX-10, 4 x 250 mm
**Eluents:** A) Water
B) Water
C) 2.0 M NaCl
D) 0.2 M Tris/HCl (pH 9)
**Gradient:** 20 mM Tris/HCl; 0 - 30 min
0.008 - 0.14 M NaCl; 0 - 30 min
0.5 M NaCl; 17 - 19 min
0.0 M NaCl; 17 - 25 min
**Flow Rates:** 1.0 mL/min
**Inj. Amt:** 50 µg (1 mg/mL)
**Detection:** 214 nm
**Samples:** HOLO (iron rich) and APO (iron poor) human transferrin samples before and after Neuraminidase treatment. Digestions were made overnight at 37°C in sodium acetate buffer at pH5.



## 4.6 Profiling Dairy Milk Caseins

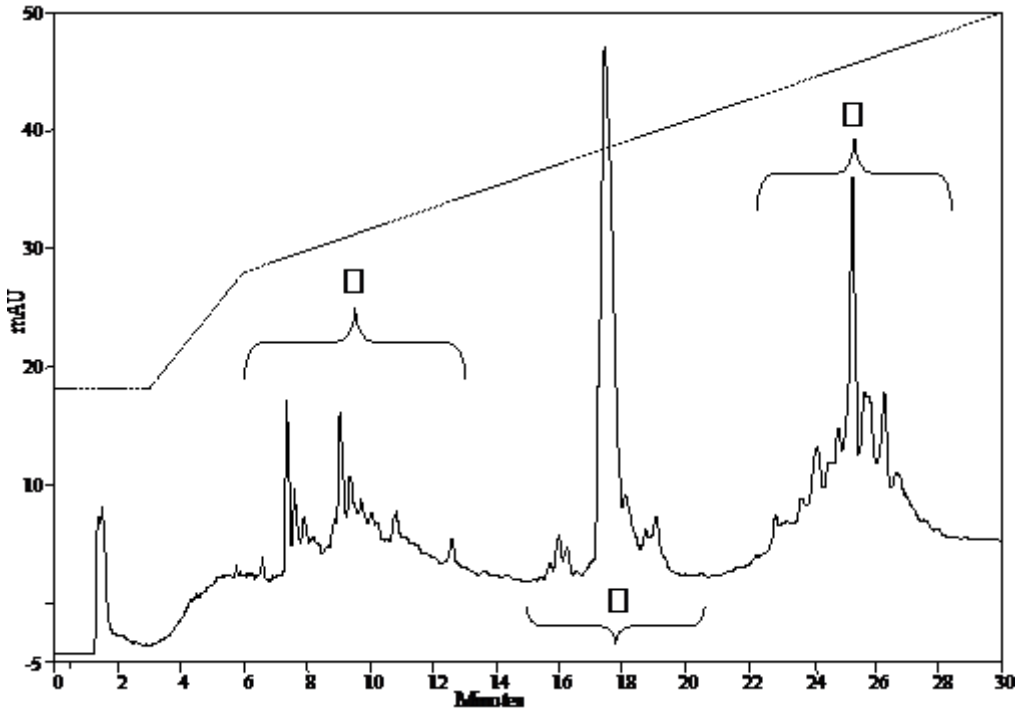
Cows milk consists of 3-3½ % proteins, 80% of which are caseins. Caseins are acidic proteins that are insoluble at their iso-electric point, pH 4.6, and exist in nature in solution as micelles. The other 20% of cows milk proteins largely consists of serum proteins; that include β-lactoglobulin A & B, α-lactalbumin, serum albumin and the immunoglobulins [5].

In the dairy industry, cows milk protein profiling is used to assess adulteration and the effects of processing. It is known that cows milk protein profiling is dependent on the species of animal as well as on the stage of lactation and the nutritional status of the animal [6]. Hence, high resolution chromatographic separations of milk proteins is useful in the regulatory monitoring of milk based products.

In this application a high resolution separation is shown for a sample of bovine caseins, including α, β and κ caseins The disruption of the micelles was achieved by dissolving the milk proteins, and running the chromatography with solvents containing urea and 2-mercaptoethanol.

| | |
|---|---|
| **Columns:** | ProPac SAX-10, 4 x 250 mm |
| **Eluents:** | A) 4 M Urea, 0.01 M 2-mercaptoethanol,<br>0.01 M HEPES, pH 7.3<br>B) 1.0 M NaCl,4 M Urea,<br>0.01 M 2-mercaptoethanol,<br>0.01 M HEPES, pH 7.3 |
| **Gradient:** | 3 min %B = 10<br>30 min %B = 35 |
| **Flow Rates:** | 1.0 mL/min |
| **Inj. Amt:** | 50 µg (1 mg/mL) |
| **Detection:** | 280 nm |
| **Samples:** | mixture of α, β & κ bovine caseins |

**Figure 9 Profiling Dairy Milk Caseins**



# 5. Troubleshooting Guide

## 5.1 Finding the Source of High System Back Pressure

A. A significant increase in the system back pressure may be caused by a plugged inlet frit (bed support) or from the instrument.

B. Check for pinched tubing or obstructed fittings from the pump outlet, throughout the eluent flow path to the detector cell outlet. To do this, disconnect the eluent line at the pump outlet and observe the back pressure at the usual flow rate. It should not exceed 50 psi (0.3 MPa). Continue adding components (injection valve, column, detector) one by one while monitoring the system back pressure. The 4 x 250 mm ProPac WAX-10 and SAX-10 should add no more than 1,500 psi back pressure at 1 mL/min. The 4 x 50 mm ProPac WAX-10 and SAX-10 columns should add no more than 400 psi (2.6 MPa) back pressure at 1 mL/min. No other component should add more than 100 psi (0.7 mPa) to the system back pressure.

C. If the high back pressure is due to the column, try cleaning (washing) the column. If the high back pressure persists, replace the column bed support at the inlet of the column.

## 5.2 Column Performance is Deteriorated

### 5.2.1 Peak Efficiency and Resolution is Decreasing, Loss of Efficiency

A. If changes to the system plumbing have been made, check for excess lengths of tubing, tubing diameters larger than 0.010 in I.D. larger than normal tubing diameter and for leaks.

B. Check the flow rate and the gradient profile to make sure your gradient pump is working correctly.

C. The column may be fouled. Clean the column using the recommended cleaning conditions in the “Column Care” section (see front page).

D. If there seems to be a permanent loss of efficiency check to see if headspace has developed in the column. This is usually due to improper use of the column such as submitting it to high backpressure. If the resin doesn’t fill the column body all the way to the top, the resin bed has collapsed, creating a headspace. The column must be replaced.

E. If the peak shape looks good, but the efficiency number is low, check and optimize the integration parameters. If necessary, correct the integration manually, so the start-, maximum- and end of the peak are correctly identified.

### 5.2.2 Unidentified Peaks Appear as Well as the Expected Analyte Peaks

A. The sample may be degrading. Proteins tend to degrade faster in solutions; therefore, store your protein samples appropriately, and prepare only a small amount of solution/mixture for analysis.

B. The eluent may be contaminated. Prepare fresh, filtered eluent. The presence of unidentified peaks on a chromatographic column can result from a myriad of causes. However, in the case of the anion exchange columns a unique source of these peak has been identified. As Tris-type buffer solutions age it has been observed that extra, spurious peak can be seen on the chromatogram, mainly during the low ionic strength portion of the gradient. It is easily possible to minimize the deleterious effects of this by making up the buffer solution regularly, by equilibrating the column and by starting the gradient at 15-20 mM of the eluting salt e.g. NaCl. This small amount of NaCl is enough to prevent the accumulation of the buffer "degradation by-product" on the column and to permit a clear blank chromatogram to be observed.

C. Run a blank gradient to determine if the column is contaminated. If ghost peaks appear, clean the column.

## 5.3 Finding the Source of High System Back Pressure

A. A significant increase in the system back pressure may be caused by a plugged inlet frit (bed support).

B. Before replacing the inlet bed support assembly of the column, make sure that the column is the cause of the excessive back pressure.

C. Check for pinched tubing or obstructed fittings from the pump outlet, throughout the eluent flow path to the detector cell outlet. To do this, disconnect the eluent line at the pump outlet and observe the back pressure at the usual flow rate. It should not exceed 50 psi (0.3 MPa). Continue adding components (injection valve, column, detector) one by one while monitoring the system back pressure. The 4 x 250 mm ProPac WAX-10 and SAX-10 should add no more than 1,500 psi back pressure at 1 mL/min. The 4 x 50 mm ProPac WAX-10 and SAX-10 columns should add no more than 400 psi (2.6 MPa) back pressure at 1 mL/min. No other component should add more than 100 psi (0.7 mPa) to the system back pressure.

D. If the high back pressure is due to the column, first try cleaning the column. If the high back pressure persists, replace the column bed support at the inlet of the column.

## 5.4 Column Performance is Deteriorated

### 5.4.1 Peak Efficiency and Resolution is Decreasing, Loss of Efficiency

A. If changes to the system plumbing have been made, check for excess lengths of tubing, tubing diameters larger than 0.010 in I.D. larger than normal tubing diameter and for leaks.

B. Check the flow rate and the gradient profile to make sure your gradient pump is working correctly.

C. The column may be fouled. Clean the column using the recommended cleaning conditions in the "Column Care" section (see front page).

D. If there seems to be a permanent loss of efficiency check to see if headspace has developed in the column. This is usually due to improper use of the column such as submitting it to high backpressure. If the resin doesn't fill the column body all the way to the top, the resin bed has collapsed, creating a headspace. The column must be replaced.

E. If the peak shape looks good, but the efficiency number is low, check and optimize the integration parameters. If necessary, correct the integration manually, so the start-, maximum- and end of the peak are correctly identified.

### 5.4.2 Unidentified Peaks Appear as Well as the Expected Analyte Peaks

A. The sample may be degrading. Proteins tend to degrade faster in solutions; therefore, store your protein samples appropriately, and prepare only a small amount of solution/mixture for analysis.

B. The eluent may be contaminated. Prepare fresh, filtered eluent. The presence of unidentified peaks on a chromatographic column can result from a myriad of causes. However, in the case of the anion exchange columns a unique source of these peak has been identified. As Tris-type buffer solutions age it has been observed that extra, spurious peak can be seen on the chromatogram, mainly during the low ionic strength portion of the gradient. It is easily possible to minimize the deleterious effects of this by making up the buffer solution regularly, by equilibrating the column and by starting the gradient at 15-20 mM of the eluting salt e.g. NaCl. This small amount of NaCl is enough to prevent the accumulation of the buffer "degradation by-product" on the column and to permit a clear blank chromatogram to be observed.

C. Run a blank gradient to determine if the column is contaminated. If ghost peaks appear, clean the column.

# Appendix A – Column Care

## B.1 New Column Equilibration

The columns are shipped in 10 mM Tris pH = 8.0 buffer containing 0.1% sodium azide. Before use, wash the column with approximately 20 mL of the starting eluent (20 min at 1 mL/min).

## B.2 Column Cleanup

**NOTE**

***When cleaning an analytical and guard column in series, move the guard column after the analytical column in the eluent flow path. Otherwise contaminants that have accumulated on the guard column will be eluted onto the analytical column.***

### B.2.1 Cleanup Solution

150  potassium nitrate in 80% acetonitrile, pH 2.0 (adjust pH with HCl)

### B.2.2 Column Cleanup Procedure

1. Rinse the column for 15 minutes with 10 mM Tris pH 8.0 before pumping the cleanup solution over the column
2. Prepare 500 mL cleanup solution.
3. Set the pump flow rate to 1 mL/min for the 4-mm I.D. columns, 0.25 mL/min for the 2-mm I.D columns, or 5.0 mL/min for the 9-mm I.D. columns.
4. Pump the cleanup solution through the column for 60 minutes.
5. Equilibrate the column(s) with starting eluent for at least 30 minutes before resuming normal operation.
6. Place the guard column back in-line before the analytical column if the system was originally configured with a guard column.

## B.3 Column Storage

### B.3.1 Short Term Storage:

For short term storage, 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 20 mM Tris pH = 8.0 eluent with 0.1% sodium azide added to avoid bacteria growth on the column.

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

## B.4 Replacing Column Bed Support Assemblies

**NOTE**

***Replace the inlet bed support ONLY if the column is determined to be the cause of high system back pressure, AND cleaning of the column does not solve the problem.***

1. Carefully unscrew the inlet (top) column fitting. Use two open end wrenches.
2. Remove the bed support. Tap the end fitting against a hard, flat surface to remove the bed support and seal assembly. Do not scratch the wall or threads of the end fitting. Discard the old bed support assembly.
3. Removal of the bed support may permit a small amount of resin to extrude from the column. Carefully remove this with a flat surface such as a razor blade. Make sure the end of the column is clean and free of any particulate matter. Any resin on the end of the column tube will prevent a proper seal. Insert a new bed support assembly into the end fitting and carefully thread the end fitting and bed support assembly onto the supported column.
4. Tighten the end fitting fingertight, then an additional ¼ turn (25 in x lb.). Tighten further only if leaks are observed.

**CAUTION**

***If the end of the column tube is not clean when inserted into the end fitting, particulate matter may prevent a proper seal between the end of the column tube end the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or break the end fitting.***

# Appendix B – References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