Agilent ZORBAX Bio Series Ion-Exchange datasheet

General Description

ZORBAX Bio Series Ion-Exchange HPLC columns were developed for use in separating and purifying biological macromolecules, including proteins, peptides and polynucleotides. These HPLC columns are based on the same zirconium-oxide stabilized packing material used in the GF-450 high performance gel filtration columns. The zirconium-oxide stabilized, 300 Angstrom silica can be used with basic mobile phases up to pH 8.5. A diol bonded phase ensures biocompatibility as well as hydrophilicity. Charged groups are covalently bound to the stabilized, hydrophilic diol surface to provide the ion-exchange character to the packing material.

Bio Series Ion-Exchange columns have a high degree of mechanical stability. Therefore, they can be used at high operating flow rates and pressures. The columns are compatible with rapid changes in ionic strength, thus short gradient times and rapid re-equilibration are possible.

The Bio Series columns are not restricted to low flow conditions. The column and packing material are stable to pressures up to 350 bar. Typical flow rates for these columns are from 1 to 6 mL/min. The higher flow rates are especially useful when re-equilibrating the column.

Columns Characteristics

The Bio Series Ion-Exchange columns are 6.2 mm ID x 80 mm in length. The columns are available with either anion exchange or cation exchange packing materials.

Name	Functionality	Description
Bio Series SAX	$-(CH_2^+N(CH_3)_3)$	strong anion exchanger
Bio Series SCX	$-(SO_3^-)$	strong cation exchanger

Each column is tested prior to shipping, to ensure efficiency. In addition, each lot of packing material is tested for stability, capacity, and protein recovery. Each packing lot must pass rigorous performance requirements, including the separation of a mixture of proteins, before the packing material is released for column production and shipment.

Several test chromatograms can be found in this guide. These test chromatograms have been generated using readily available samples. It is recommended that you test your new columns prior to use with these reference separations to verify column performance and retest periodically thereafter.

Safety Considerations

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users of liquid chromatographic equipment should be aware of the toxicity or flammability of their mobile phases.
- Because of its small particle size, dry ZORBAX packing is respirable. Columns should only be opened in a well-ventilated area.

Operational Guidelines

- The direction of flow is marked on the column.
- While it is not harmful to the column, reverse flow should be avoided except to attempt removal of inlet pluggage (see “Column Care” section).
- A new column is shipped in buffer which contains sodium azide. Initially, care should be taken not to pass any mobile phase through the column that might cause a precipitate.
- ZORBAX Bio Series Ion-Exchange is compatible with water and all common organic solvents.
- The use of a guard column is recommended to protect the ZORBAX Bio Series Ion-Exchange column and extend its useful lifetime.
- Avoid use of this column below pH 2.5 or above pH 8.5.
- Maximum operating pressure for Bio Series Ion-Exchange columns is 350 bar (5000 psi).
- Maximum operating temperature is 80ºC.

Mobile Phase Selection

There have been several good reviews published on the subject of ion-exchange mobile-phase selection (Ref. 1-6). Before beginning a particular separation, a review of the relevant literature is recommended. Several mobile-phase factors must be considered. These considerations include: mobile-phase strength, composition, compatibility with the sample, and pH.

Ion-exchange chromatography usually involves gradient elution. This technique often requires that the sample be applied to the column in a weak buffer. This allows the sample to be strongly held on the column. Sample components are eluted with a gradual change of mobile phase, to a stronger buffer. The stronger buffer may contain a high ionic strength of the buffer used for the weak buffer, or contain a higher concentration of another salt. Alternatively, the stronger eluting buffer may be of a different pH than the weak buffer. In many cases, a combination of a change in both pH and ionic strength can be used.

In some HPLC ion-exchange methods, detergents are used in the mobile phase. Ion-Exchange columns may be used with detergents and other denaturating solvents, including most organic solvents. In general, anionic detergents should not be used on the SAX column, and cationic detergents should not be used on the SCX column.

Although halide salts are often used in ion-exchange chromatography, they are corrosive to stainless steel. Alternatives to halide salts include phosphate, acetate, and sulfate salts. We strongly recommend that, if halide salts are used, they be thoroughly flushed from the HPLC system after use. The columns should not be stored in mobile phases which contain halides.

All solvents should be filtered and degassed prior to use. Whenever possible, use HPLC or analytical-grade solvents and chemicals.

Column Equilibration

The column is supplied in buffer which contains sodium azide as an antibacterial agent. After attaching the column to the chromatographic instrument, wash the column with 20 mL of starting buffer. Change the buffer to the higher strength buffer (i.e., 1.0M buffer or salt) and flush an additional 20 mL of liquid through the column. Re-equilibrate the column with 20 mL of starting buffer. The column should now be ready for use.

Sample Introduction

Samples can be injected using a continuous-flow injection device. Standard loop injectors on most HPLC systems are suitable devices. Alternatively, samples may be pumped directly onto a pre-equilibrated column. Samples should be free of any particulates (filter, if necessary).

The amount of sample which can be injected is highly dependent on sample composition. Although actual capacity may vary, the protein capacity for these columns is approximately 1-5 mg/column.

Storage Recommendations

When the column is in frequent use, it is not necessary to flush out the mobile phase daily, although care should be taken to avoid potential bacterial growth. If the column will not be used for several days, it is advised that the mobile phase be flushed out and replaced with one containing an antimicrobial agent (e.g., sodium azide).

Do not store the column in a mobile phase which contains halides or detergents. A recommended long-term storage condition is 0.1 M Bis-Tris, pH adjusted to 6.5, containing an antimicrobial agent (0.005% sodium azide). Do not store without an antimicrobial agent. Alternatively, flush and store the column with a water/methanol mixture.

After filling the column with an appropriate storage liquid, remove the column from the HPLC instrument, and seal ends with protective screw fittings used during shipping.

Columns should be stored at room temperature.

References

1. M. Hearn, F. Regnier, C. Wehr, *Amer. Lab.*, Oct. **1982**, 18.
2. F.E. Regnier, *Science*, **1983**, 222, 245-252.
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4. R.W. Stout, S.I. Sivakoff, R.D. Ricker, H.C. Palmer, M.A. Jackson, T.J. Odiorne, *J. Chromatogr.*, **1986**, 352, 381-397.
5. F.E. Regnier in *Methods in Enzymology, Enzyme Purification and Related Techniques*, Part C, Vol. 104, Academic New York, **1984**, 170-189.
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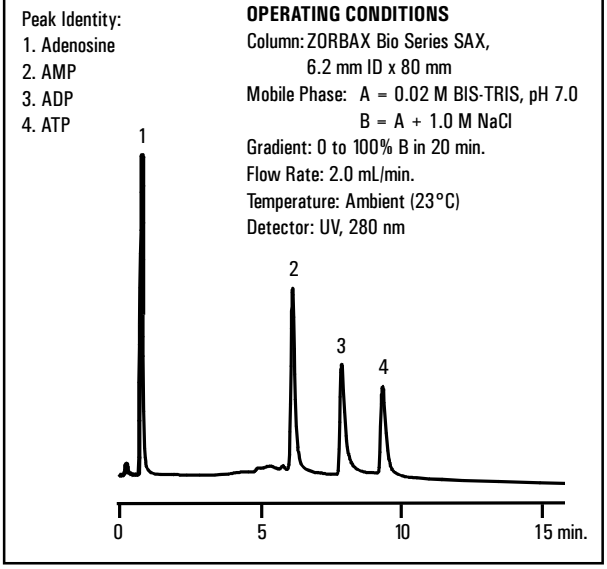
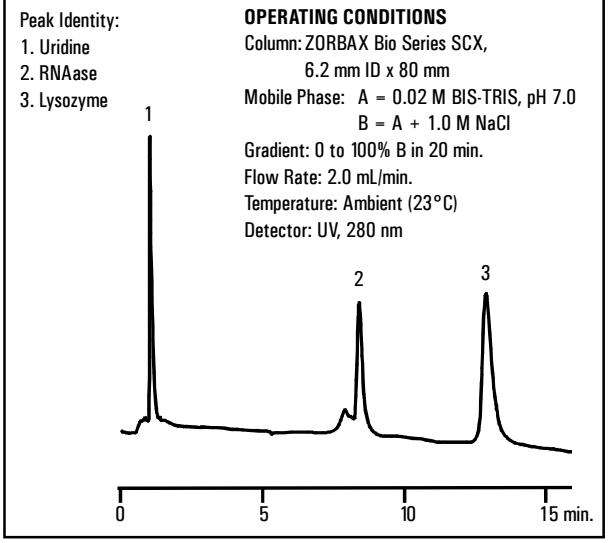
Separation of Adenosine, AMP, ADP and ATP on ZORBAX Bio Series SAX



Uridine, RNAase, & Lysozyme on ZORBAX Bio Series SCX



Ordering Information

	Agilent Part No.
ZORBAX Bio Series Ion-Exchange Columns	
6.2 mm ID x 80 mm	
Bio Series SAX	820944-903
Bio Series SCX	820944-904
Ion-Exchange Cartridge Guard Columns (4 pack)	
4.6 mm ID x 12.5 mm	
SAX Guard Column	820950-903
SCX Guard Column	820950-904
Guard Column Hardware Kit	820777-901

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