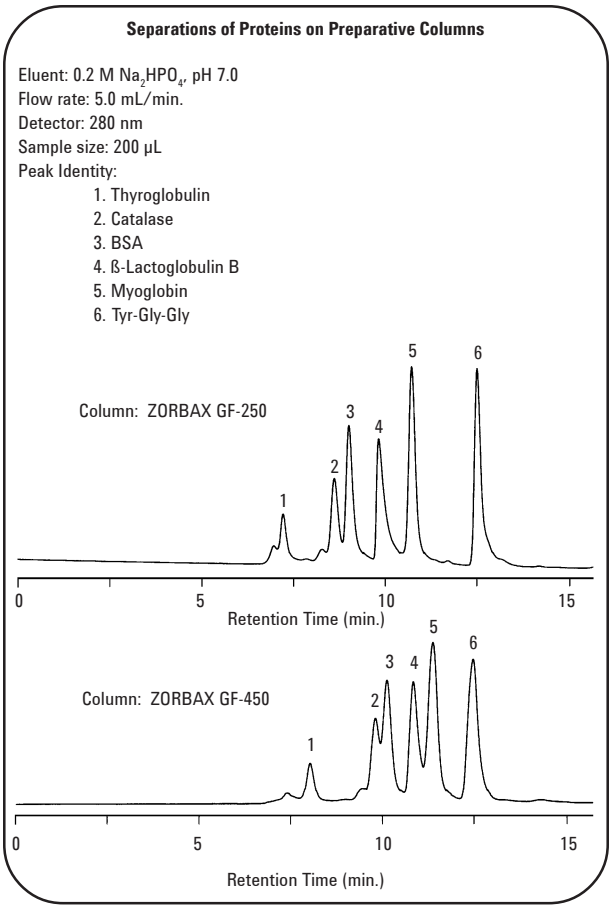
Agilent Zorbax PrepHT GF-250 and GF-450 datasheet

General Description

Zorbax PrepHT GF-250 and GF-450 are surface-stabilized, hydrophilic gel filtration columns useful for the size separation of biological macromolecules. These columns have been specifically designed to provide superior efficiency, pH stability, and operational lifetime when using typical aqueous buffer solutions (pH 3.0-8.5) as the mobile phase.

The GF-250 and GF-450 columns separate compounds by gel filtration (exclusion). Separations depend on the size of the sample molecules and the effective pore diameter of the packing material. Typically, the elution order or retention time obtained for a given molecule is inversely proportional to the logarithm of its molecular size. Molecular size is related to the molecular weight. Very large molecules are excluded from the pores, have the shortest path through the column, and elute first. Medium size molecules partially diffuse into the pores, and elute later. The smallest molecules, which can totally permeate the pore volume of the packing, elute last.

The GF-450 and GF-250 columns are complimentary. The GF-450 column provides high performance gel filtration separation of high molecular weight biomolecules which are excluded from the linear separation range of the smaller pore sized GF-250 column. The optimal separation ranges for the columns are 900,000 to 25,000 Daltons (D) for the GF-450 column and 250,000 to 10,000 D for the GF-250, assuming spherical macro-molecules. (See Figure 1)



Column Performance

Column Quality Control

Each packing lot is thoroughly tested prior to use for column manufacture. This test includes a separation of a standard protein test mixture. When calibrating your column, use a freshly prepared protein mixture of commercially available lyophilized proteins. Measure column efficiency using a small-molecule, permeation peak, e.g., sodium azide. Prepare samples by dissolving protein and/or sodium azide in mobile phase collected from the detector waste effluent. Using this liquid eliminates refractive index disturbances in the chromatogram.

Stability

The hydrolytic stability of this product was determined by continuously pumping 0.1M $(NH_4)_2SO_4$, 0.05M Tris, 0.005% sodium azide, pH 8.25 ± .05, for three weeks, while evaluating the column's separation performance. This test corresponds to 4-6 months of heavy use. At high pH (above 8.5) column life will likely be reduced.

Recovery

Protein recoveries from the GF-250 and GF-450 columns are typically 85-100% of the total protein applied to the column. This recovery level is dependent on the protein in use and the mobile phase selected. Typically, biological activity of macromolecules is fully retained, assuming mobile phase compatibility with the biological substance.

Safety Considerations

Some important points to keep in mind for safe operation with LC components:

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users of liquid chromatographic equipment should be aware of the potential hazards from such leaks due to the toxicity or flammability of the chosen mobile phase.

- Because of its small particle size, dry Zorbax packing is respirable. Columns should only be opened in a well ventilated area.
- These columns have not been approved for use in processing products for human use.

Operational Guidelines

Installation

- Assemble the column by threading the end fittings onto both ends of the cartridge column and hand tighten them.
- Attach the column inlet to the appropriate port on your injector system. There is an arrow on the column to indicate the direction of flow of the mobile phase.
- All components should be coupled as closely as possible. Dead volume, excess mixing volumes, and extra lengths of tubing should be minimized. The use of low dead volume connectors is advised.
- The column is shipped in methanol. Flush the column with water before use with buffers.
- Check the mobile phase for sample solubility and biocompatibility prior to use. All solvents should also be filtered and degassed prior to use.
- The recommended pH limits for these columns are pH 3 to 8.5. Use of the columns outside these limits will reduce expected column life, but can be tolerated.
- Zorbax GF-250 and GF-450 columns may be operated at temperatures up to 40°C and at any lower temperature which does not induce the formation of solids in the mobile phase. The system back pressure will increase markedly near the freezing point of the mobile phase, as a result of increasing viscosity.

Mobile Phase Selection

For biological separations, the choice of mobile phase is critical and must consider both column performance and the maintenance of biological function. Typical buffers used for classical protein gel filtration are acceptable for the GF-250 and GF-450 columns. These include denaturing buffers and those containing detergents.

All silica-based gel filtration columns possess a slight negative charge. This charge is likely due to unreacted silanol groups on the silica surface. The GF-450 column has a slightly higher negative charge density than some other silica-based columns, primarily due to the zirconia stabilization process. This slight negative charge does not affect the separation of biomacromolecules when using typical buffers of 0.1-0.5 molarity, and pH levels of 5.0-8.5. At low pH (4.0) and low ionic strength (less than 0.05M), an ion exchange effect may be noticed. This effect can be eliminated by either raising the pH or the ionic strength of the buffer. *An example of a routinely used buffer is 0.2M sodium phosphate, pH 7.0.* Using buffers with pH values above 8.5 does cause a slow base-catalyzed dissolution of the silica packing, but can be tolerated for short periods at the expense of somewhat reduced column life. Buffers with pH values below 5.0 should be avoided, not because of any deleterious effect they have on the packing, but because of possible alteration of the samples of interest. These sample alterations may adversely affect the expected separations. The column itself is stable to pH 3.0. The use of an antimicrobial agent in the mobile phase is also recommended (e.g. 0.005% sodium azide).

The GF-250 and GF-450 columns are compatible with most organic solvents.

Column Care

To protect the GF-250 and GF-450 columns and increase column life, we recommend the use of a guard column inserted between the injector and the column to protect against particulates in the sample.

Halide salts, such as sodium chloride, are corrosive to steel. If it is necessary to use such salts, thoroughly flush the HPLC system after use. Store the column in a mobile phase which does not contain halides.

If the column becomes plugged, try to clear the blockage by backflushing the column.

If the simple backflushing is not successful, back-flush the column with 30% isopropanol; 30% isopropanol with 1mM EDTA (pH 4.5); or 50% acetonitrile, containing 0.05% TFA. This last cleaning procedure is quite stringent and may restore column performance.. If not, you can assume that the column has been compromised beyond repair.

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 buffers be flushed and replaced with water containing an antimicrobial agent (e.g., sodium azide) or with 100% organic solvent.
- Do not store the column in a mobile phase which contains halides.
- Storage in 100% methanol, ethanol, or acetonitrile is recommended.
- Remove the column from the HPLC instrument and seal the end-fittings with screw plugs. If the end-fittings are removed, cover the ends using the plastic caps used to ship the columns.
- Columns should be stored at room temperature.

Agilent Ordering Information

For more information on our products, visit our Agilent Technologies home page on the World Wide Web at: www.agilent.com/chem/supplies
For Technical Support in the US and Canada, call 1-800-227-9770 or call your local Agilent sales office.

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