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Proteomix HIC Phases

Column Information

Proteomix® HIC columns are specially designed for high resolution and high efficiency separations of proteins, oligonucleotides and peptides. Utilizing proprietary surface technologies, *Proteomix*® HIC-NP resin is made of non-porous polystyrenedivinylbenzene (PS/DVB) beads with narrow-dispersed particle size distribution. As shown in **Figure 1**, the PS/DVB bead is modified with alkyl groups or aryl group that provides hydrophobic interaction with analytes. *Proteomix*® HIC-NP resin is highly rigid and mechanically stable. In comparison to silica based HIC phase media, *Proteomix*® HIC-NP phases have advantages for biomolecule separations with wide pH range (2-12) and high thermal stability. The nonporous structure and narrow particle distribution offer special selectivity, high resolution separation of proteins such as mAb (monoclonal antibody), ADC (antibody drug conjugate) and related protein fragments, DNA and oligonucleotides. *Proteomix*® HIC-NP media is applicable at laboratory discovery, laboratory-scale purification and process chromatography for the production of a few mgs to kilogram of proteins.

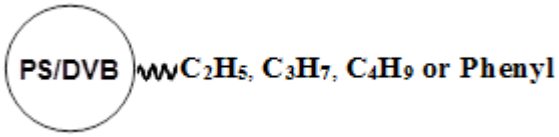

Figure 1. Surface features of *Proteomix*® HIC resins

Column Stability and Performance

Proteomix® HIC columns are based on PS/DVB resin and all the surface coatings are chemically bonded onto PS/DVB support, which allows exceptional high stability. The columns are compatible with most aqueous buffers, such as ammonium sulfate, sodium acetate, phosphate, Tris and a mixture of water and acetone, methanol, acetonitrile and THF. When 25 mM sodium phosphate buffer at pH 7.0 was used as the mobile phase to run *the Proteomix* HIC, 400 injections or 3 months of usage has negligible deterioration for the columns. Since the surface chemistry is built with highly controlled process, the *Proteomix*® HIC columns has high lot to lot consistency, as shown in **Figure 2** of ADC separation from 3 lots of HICButyl NP5 columns.

Technical Specifications

Resin Matrix	Spherical, highly cross-linked PS/DVB
Pore Size:	Nonporous
Particle Size:	1.7 µm and 5 µm
Phase Structure:	Ethyl, Propyl, Butyl and Phenyl (5 µm) Ethyl and Butyl (1.7 µm)
Separation Mechanism	Hydrophobic interaction
pH Stability	2-12
Operating Temperature	Up to 80 ˚C
Operating Pressure limit	6000 psi (5 µm) 8,000 psi (1.7 µm)
Mobile Phase Compatibility	Compatible with aqueous solution, a mixture of water and acetonitrile, acetone, methanol, or THF

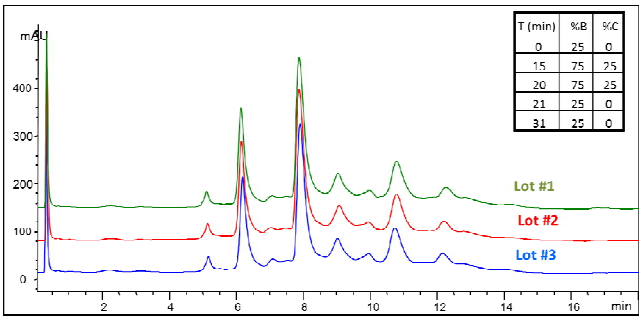

Figure 2. Columns: *Proteomix*® HICButyl-NP5 (5 µm, 4.6 x 35 mm); Mobile phase: A) 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0, B) 0.025 M sodium phosphate pH 7.0, C) 100% IPA; Flow rate: 0.8 mL/min; Detector: UV 214 nm; Column temperature: 25 ℃; ADC, 1.0 mg/mL in 25 mM sodium phosphate; Injection: 10 µL.

Safety Precaution

Proteomix® HIC columns are normally operated under high pressure. Loose connections will cause leaking of buffers and injected samples, all of which should be considered as the hazards. In the case of leaking, proper gloves should be worn for handling the leaked columns. When open the columns, proper protections should be used to avoid inhalation of the small polymer particles.

Column Installation and Operation

When a column is shipped or not in use, it is always capped at both ends. When install the column to the system, first remove the end caps. Make the flow direction as marked on the column. Unless a user has special purpose to reverse the flow direction, for example, removal of the inlet pluggage, follow the flow direction as labeled. Column connections are an integral part of the chromatographic process. If ferrules are over tightened, not set properly, or are not specific for the fitting, leakage can occur. Set the ferrules for column installation to the HPLC system as follows:

(a) Place the male nut and ferrule, in order, onto a 1/16" O.D.

piece of tubing. Be certain that the wider end of the ferrule is against the nut.

(b) Press tubing firmly into the column end fitting. Slide the nut and ferrule forward, engage the threads, and fingertighten the nut.

(c) While continuing to press the tube firmly into the endfitting, use a 1/4" wrench to further tighten.

(d) Repeat this coupling procedure for the other end of the column.

Samples and Mobile Phases

To avoid clogging the column, all samples and solvents including buffers should be filtered through 0.45 μm or 0.2 μm filters before use. It is also strongly recommended to use a pre-column filter (0.5 μm frit) or a guard column to protect the column. *Proteomix*® HIC columns are compatible with aqueous mobile phases or a mixture of organic and water, such as methanol or acetonitrile and water. Typical eluents contain ammonium, sodium and potassium salts of sulfate, phosphate, chloride, acetate, or Tris. Always use an inline degassor or degas the mobile phase prior to use. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum

Since the HIC chromatography mainly depends on the hydrophobic interaction of analytes with the solid phase, sodium phosphate buffer with and without sodium sulfate is a primary choice of buffers in the reversed salt gradient. Sometimes, resolution can be improved with some organic modifiers (the content of organic solvents should not exceed 30%). A typical example is shown in **Figure 2**.

Column Care

Shipping Solvent: *Proteomix*® HIC columns are shipped with 25 mM sodium phosphate, pH 7.

Column Storage: It is recommended to store columns at room temperature in water or 25 mM sodium phosphate, pH 7.

First-time Use: During stocking and shipping, the packing could be dried out. It is recommended to wash the column at a low flow rate such as 0.1 mL/min, followed by 10-20 column volume of the running buffer to activate the column, until the baseline is stabilized. If the column backpressure and baseline fluctuate, this might be due to the air bubbles trapped inside the column. Flush the column with higher flow rate for 2-5 minutes, for example 1.5 mL/min for a 5 μm, 4.6 x 3.5 mm column. If the mobile phase or pH is quite different from the stock buffer in the column, it is recommended that the column be washed first with the new mobile phases for 10 column volume.

Recommended Flow Rate: 0.1-1.5 mL/min. A high flow rate can be applied as long as the back pressure does not exceed the maximum pressure limit.

Solvent Exchange: If there is a need to use organic additives or organic solvents as buffer, care should be taken to avoid salt precipitation. The column should be washed with 2 column volumes of water between the salt buffers and organic solvents. During the exchange of the solvents, backpressure may fluctuate. A low flow rate should be applied to make sure the back pressure not exceeding the pressure limit.

CIP: Columns can be cleaned with 1 volume of 25 mM NaOH, 3.0 M urea or guanidinium chloride. Organic solvents such as THF, IPA, methanol, ethanol, acetonitrile also can be used to clean hydrophobic deposit. Then the columns should be washed with enough water before switching back to the regular buffers.

Column Protection

To extend the column life, it is necessary to filter both sample and mobile phases daily with 0.2 μm filters, especially for sub 2 μm columns. To further block the residual particulates in the sample or the mobile phase entering into columns: one of the following two additional measures are recommended:

The 1st recommendation is to install a guard column, 2.0 x 10 mm or 4.0 x 10 mm. It will more effectively trap highly adsorptive sample components and residual particulates in the sample, the mobile phase or from the HPLC system. After the use of a guard column for certain period of time, it is recommended to back flush the guard column daily with saline salt (1 M NaCl in 25-100 mM sodium phosphate pH 7) for 10 column volumes.

The 2nd recommendation is to install a precolumn filter with a cut off <0.3 μm. It is required to change and replace the filter, any time the back pressure is built up or the column performance is decreased. It is recommended to back flush the column with saline salt (1 M NaCl, or phosphate buffer) for 10 column volumes, each time the precolumn filter is cleaned or replaced.

Order Information

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