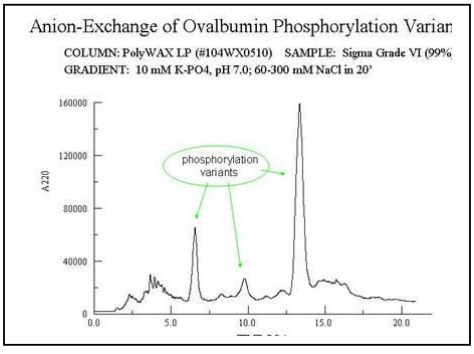
PolyWAX LP™

- for Anion-Exchange of Proteins and Nucleic Acids



PolyWAX LP™ for:

1. Isocratic separation of amino acids, peptides, and proteins.
2. Selective isolation and separation of phosphopeptides.

CLICK HERE for details!

Most proteins have isoelectric points below 7, and are best purified or analyzed by anion-exchange chromatography. PolyWAX LP™ is a hydrophilic weak anion-exchange (WAX) material developed by PolyLC for HPLC of enzymes and other proteins. Selectivity is excellent, with high or quantitative recovery of applied biological activity. Most anion-exchange materials based on polyethyleneimine (PEI) are prepared with the conventional branched polymer. PolyWAX LP™ is prepared with linear PEI, which confers greater selectivity and recovery. The following example demonstrates the ability of PolyWAX LP™ to separate proteins differing by a single phosphate group.

Anion-exchange is the method of choice for resolution of oligonucleotides and their analogs > 15 bases. It is also much faster and convenient than PAGE gels for purification of the double-stranded DNA products from PCR reactions.

Use PolyWAX LP™ for:

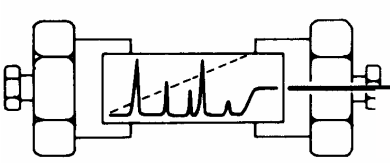
1) Purification of acidic proteins and polypeptides from natural products.

2) Analysis and purification of oligonucleotides and their analogs as well as amplified PCR products.

3) In proteomics, predigest fractionation of intact proteins via mixed-bed ion-exchange.

4) Anion-exchange of small, acidic solutes.

For small solutes, use our 100-Å material. For peptides, use 300 Å. For proteins > 20 KDa, we recommend pore diameters of at least 1000 Å for optimal selectivity and efficiency. Our 3-µm, 1500-Å material affords superior separations of closely-related protein variants.



PolyLC INC.

PolyWAX LP™ Columns

Initial Use: PolyWAX LP™ is a silica-based material with an adsorbed, cross-linked coating of linear polyethyleneimine. It is a weak anion-exchange (WAX) material. The capacity is high for both small and large solutes. Columns are shipped filled with methanol. Flush new columns with at least 15 column volumes of water (30 ml for a 200 x 4.6-mm) then condition with a salt solution prior to initial use. The hydrophilic coating imbibes a layer of water of hydration and swells, especially in the presence of salt solutions. This causes a modest, irreversible increase in the column backpressure. Since the swelling increases the surface area of the coating, this increases the binding capacity for proteins as well. This process should be hastened by eluting the column with a concentrated salt solution for two hours prior to its initial use. A convenient solution for the purpose is 0.2 M NaH_2PO_4 + 0.3 M sodium acetate. Alternatively, if the column is to be used for proteins or peptides that may interact with heavy metals (e.g., phosphopeptides), then the column should be passivated by elution for 20-24 hours with 40 mM EDTA.2Na at a low flow rate.

New HPLC columns sometimes absorb small quantities of proteins or phosphorylated peptides in a nonspecific manner. The sintered metal frits have been implicated in this. Eluting the column for 20-24 hr. at a low flow rate with 40mM EDTA.2Na usually solves the problem. This passivates all metal surfaces in the HPLC system, as well as the column [CAUTION: This treatment can affect the integrity of the frits in some cases, and should probably be avoided with columns packed with 3-µm material. In some cases this has also caused the collapse of 5-µm, 200-Å column packings].

Routine use: Columns should be used at ambient temperatures. Filter mobile phases and samples before use. Failure to do so may cause the inlet frit to plug. If a salt gradient is being used, flush the column with 15 ml of the high-salt buffer before equilibrating with the low-salt buffer. At the end of the day, flush the column with 15 column volumes of water and plug the ends.

Loading Capacity: The loading capacity of a 4.6mm ID column is about 4-5 mg of protein/injection, depending on the strength of the protein's binding to the support.

Storage: 1) Overnight: 100% mobile phase A. 2) Several days: Store in water. 3) Longer periods: Store in water in the refrigerator, with the ends plugged. **ACN can be added to the storage solvent (e.g., ACN:Water = 80:20) to retard microbial growth.**

Column maintenance:

1) After several hundred operating hours, reverse the direction of flow and elute the column for several hours with a high-salt buffer. Continue using the column in the reverse direction.

2) Silica-based columns are slowly degraded at basic pH. Transient exposure to > pH 8.0 should have minimal effect on the column. If prolonged use above pH 8.0 is expected, then inserting a saturator column of inexpensive silica in-line before the injector valve will prolong column lifetime.

Anion-exchange of peptides: In contrast to proteins, acidic peptides can be run at lower pH down to the range where Asp- and Glu- residues start to lose charge (@ pH 4). Retention reaches a maximum around pH 4.5. Peak shape and selectivity can sometimes be improved by inclusion of 10% organic solvent (MeOH or ACN) in the mobile phase.

Anion-exchange of proteins: This is typically performed in the pH range 7.0-8.0, using either increasing salt or decreasing pH gradients. The higher the pH, the stronger the binding of proteins. Tris is a good buffer in this range. Since halide salts tend to corrode stainless steel, acetate salts are often used to generate the gradient. However, it may be necessary to use a chloride salt gradient if the absorbance is to be monitored below 235 nm. If so, flush with 15 column volumes of water at the end of the day to preclude corrosion. It is also helpful to passivate the column every 3-4 weeks with EDTA.2Na (@ above) if chloride salts are used with regularity.

- For proteins < 15 KDa, a 300-Å material can be used. For larger proteins, a 1000-Å column is recommended.

Anion-exchange of oligonucleotides and oligonucleotide analogs: For oligos < 25 bases long, use 300-Å material. For oligos > 25 bases long and dsDNA, use of a 1000-Å column will afford elution without excessive amounts of salt. It is helpful to include 25-50 % acetonitrile or propanol in both mobile phases. Large oligos may require over 1 M salt for elution. It is advisable to use a chaotropic salt (*e.g.*, NaBr or $NaClO_4$) for the gradient in cases where there could be a tendency for self-association like GC-rich sequences; otherwise, ammonium acetate can be used.