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Instructions for Mixed-Bed IEX Columns of PolyCAT A® and PolyWAX LP®

Unless stated otherwise, the mixed-bed columns contain equal amounts of PolyCAT A® (a WCX material) and PolyWAX LP® (a WAX material) and have pore diameters of 1000 Å. Both materials are silica-based.

Initial Use: Mixed-bed columns are packed with equal amounts of the two materials unless stipulated otherwise. Columns are shipped in 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. A convenient conditioning procedure is to equilibrate the column with the low-salt buffer for 30 minutes, then run three gradients in succession using the buffers intended for protein applications, with thorough reequilibration (30-35 minutes) between gradient runs. If undue sensitivity to heavy metals is anticipated, then the columns can alternatively be conditioned with 40 mM EDTA.2Na (filtered, but pH not adjusted) at a low flow rate for 20-24 hours.

Routine Use: Proteins can be eluted from mixed-bed IEX columns with salt and/or pH gradients.
Cell lysates: A pH of 6.0 seems to afford good distribution of the proteins in cell lysates, either mammalian or microbial. MES (morpholinoethanesulfonic acid) buffers well in this range. Mobile Phase A: 10 mM MES, pH 6.0. Mobile Phase B: 10 mM MES + 0.8 M NaCl, pH 6.0. This mobile phase permits absorbance to be monitored at 220 nm. NaClO4 can be substituted for NaCl if protein solubility is a problem and it's not important to recover the proteins in a biologically active form (NaClO4 is a chaotropic salt and tends to denature proteins). Modest amounts of organic solvent (*e.g.*, 5% methanol or acetonitrile) can be included in the buffers if desired. An alternative is to use ammonium acetate as both the buffering and gradient salt, running a gradient from 10-800 mM. This mobile phase is entirely volatile but absorbance can only be monitored at 270 or 280 nm.
Serum or plasma: A pH of 7.0 seems to distribute the proteins more uniformly throughout the gradient with these samples. HEPES can be substituted for MES as the buffer. Again, ammonium acetate can be used as both the buffering and gradient salt.

An alternative elution method is to bind in 15 mM ammonium acetate, pH 6, then run a gradient to 20% acetic acid in water. This uncharges the carboxyl- groups in both proteins and on the PolyCAT A material, leading to elution of proteins in a quite volatile solvent. This approach should be considered experimental.

Gradient schedule: Try the following: 0-10', 0-12% B; 10-30', 12-60% B; 30-40', 60-100% B. Flow rate: 1 ml/min for a 4.6-mm i.d. column, 0.2 ml/min for a 2.1-mm i.d. column, etc. Reequilibrate for 35' between runs.

Collecting fractions for proteomics: If nonvolatile salts are being used, then it's convenient to collect the fractions in the wells of a dialysis plate and then immerse the bottom of the plate in a solution such as 50 mM ammonium bicarbonate that's suitable for the next step. Try to catch proteins of particularly high abundance in their own individual fractions. The more fractions collected at this stage, the lower the abundance of proteins that can be identified.

Avoid prolonged exposure to pH > 8.0. Use ambient temperature (20-25°C), as the polypeptide-based coating of PolyCAT A is more sensitive to elevated temperatures than are other materials. Filter mobile phases and samples before use. Failure to do so may cause the inlet frit to plug. If samples are particularly dirty, then it's a good idea to attach a guard cartridge of the same materials (*e.g.*, item# JGCCTWX0510, J22GCCTWX0510, etc.). Make at least one blank gradient run before injecting the first sample of the day. 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 mg of protein/injection, depending on the strength of the protein's binding to the support.

Storage: 1) 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: After every 250 runs, invert the column and run it backwards overnight, at a low flow rate, with 40 mM EDTA.2Na. Continue using the column in this inverted direction for the next 250 samples, then repeat this treatment.

Minimize Iron in the System: Cation-exchange materials such as PolyCAT A® chelate Fe^{+3}, which ruins their performance. If chloride-containing mobile phases are used regularly, passivate the column and the HPLC system every 4 weeks with the 40 mM EDTA.2Na solution as described above. NOTE: If the HPLC system has not been used for several days (e.g., over a weekend), then Fe^{+3} ions tend to accumulate in the fluid in the lines. When restarting the system, flush this fluid to waste offline before diverting flow through the column.