

Care and Use Guide for Discovery BIO PolyMA-WAX

Discovery BIO PolyMA-WAX was developed for weak anion-exchange separations of proteins and peptides. The packing material comprises 5µm mono-disperse polymethacrylate particles functionalized with diethylaminoethyl groups. High efficiency and recovery are two characteristics of the Discovery BIO PolyMA-WAX column.

Discovery BIO PolyMA-WAX product specifications:

Particle composition: spherical polymethacrylate
Functional group: diethylaminoethyl, weakly basic ion-exchange
Counter ion: Cl⁻ (chloride)
Bed volume: 0.83mL
Particle size: 5µm mono-disperse
Pore size: 1000Å
Concentration of ion exchange groups: 0.3meq/g
pH range: 2 – 11
Temperature range: 4 – 50°C
Maximum pressure: 735psi (4.9Mpa, 50kg/cm²)
Maximum flow rate: 1.5mL/min (do not exceed the maximum pressure)
Maximum sample injection: < 10mg per injection
Shipping solvent: 0.05% NaN₃ in water

Care – Recommendations to maximize column life

We recommend you reproduce the test chromatogram supplied with the column. If your results deviate significantly, please contact our technical services for some troubleshooting suggestions. Routinely retesting your column throughout its lifetime will also make sure you are developing the best possible methods.

Protection: Use HPLC grade water. Filter samples and mobile phases with a 0.45µm or smaller porosity filter, and be sure buffer precipitation does not occur upon mixing mobile phase components. Always use fresh mobile phase and prevent or be alert for microbial growth. Use an in-line filter (0.45µm) between the injector and column to catch particulates. Minimize pressure surges. Do not drop or physically shock the column.

Column flushing and storage: For overnight storage, the column should be flushed with 10 column volumes of a pH 6 to 7 buffer. Do not store the column in solutions below pH 3 or above pH 10. For longer-term storage, flush the column with 10 column volumes of a pH 6 to 7 buffer, followed by 10 column volumes of water. We recommend storing the column in a solution of 0.05% NaN₃ to prevent microbial growth. All columns and HPLC systems should be rinsed with water to prevent corrosion. Columns should be stored between 15°C and 30°C. During storage, avoid exposing the column to direct sunlight.

Cleaning and regeneration: Loss of efficiency or resolution, poor peak shape, and increasing back pressure are indicative of column degradation or fouling. If this occurs, we recommend the following cleaning procedure. Check performance after each step. First, inject up to 500µL of 2.0M NaCl in water 5 or 6 times. Second, inject up to 500µL of 0.1M NaOH in water 5 or 6 times. Rinse and equilibrate the column. Third, inject up to 500µL of 5M acetic acid in water 5 or 6 times. Rinse and equilibrate the column. If these steps fail to improve performance, please call our technical services.

Use – Recommended operating conditions

Column equilibration: Flush new or stored columns with 10 column volumes of the highest concentration of salt in your mobile phase gradient. After equilibration, condition with 10 column volumes of the initial buffer of the gradient.

Salts and Buffers: Typical mobile phases are gradients of increasing salt (NaCl) in a buffer compatible with your protein. Proteins elute when the salt concentration equals their isoelectric point where they have a net zero charge. Use any buffer compatible with your protein on the Discovery PolyMA-WAX column.

pH: An eluent of pH of 2 to 11 can be used. Do not store the column below pH 3 or above pH 10.

Organic solvents: Although Discovery PolyMA-WAX particles can withstand high percentage of organic in the mobile phase, it is important that you keep the organic concentration below the point where your proteins and buffer salts precipitate. Very hydrophobic proteins and peptides may require organic mobile phase additives to solubilize and / or to elute them from the PolyMA column. Ethylene glycol, 6M urea, CHAPS, CHAPSO, and glycerol are examples. Acetonitrile and methanol may be added to improve the separation, especially with peptides. Because they affect solubility of salts, when adding any organic modifier to the mobile phase, monitor increases in the system pressure that may indicate salt (buffer) precipitation. To remove strongly-bound compounds, follow the cleaning and regeneration instructions described above.

