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# *Proteomix*® AAV SAX Phase

## Column Information

*Proteomix*® AAV SAX columns, specially developed by Sepax Technologies, are engineered for superior performance in separating empty and full adeno-associated virus (AAV) capsids. These columns feature a monosized 3 µm spherical resin matrix, made from highly cross-linked poly(styrene-divinylbenzene) (PS/DVB). The bead is modified with a nanometer-thick hydrophilic, neutral polymer coating to reduce non-specific interactions with biological analytes. The surface of the resin is further bonded with a uniform layer of trimethylammonium using Sepax's proprietary chemical attachment methods, offering high ion exchange capacity and selectivity, as shown in **Figure 1**. The incorporation of the strong anion exchanger with quaternary ammonium functional groups facilitates effective sample binding and narrow band broadening by reducing secondary interactions. Such unique surface engineering ensures superior resolution, efficient separation, and high recovery in AAV analyses.

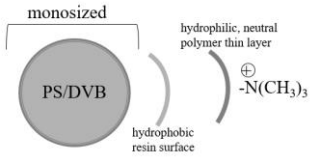


**Figure 1**. Chemical compositions of *Proteomix*® AAV SAX phase.

## Column Stability and Performance

*Proteomix*® AAV SAX columns are of high mechanical strength, resin packing stability, as well as chemical stability. This unique resin architecture achieves unparalleled resolution and efficiency in separating empty and full AAV capsids. Shown in **Figure 2** is a typical chromatogram for separating the empty and full capsid of AAV8 mixture on a 4.6 × 50 mm, *Proteomix*® AAV SAX column.

*Proteomix*® AAV SAX columns are designed to tolerate high pressures, offering flexibility to adapt and optimize operational conditions to meet specific application requirements. Additionally, these columns are compatible with multi-angle light scattering (MALS) detectors, ensuring low noise levels and enhancing their suitability for a wide range of applications that require accurate molecular weight determination. Furthermore, an AAV8 standard sample combined with UV-MALS was employed for each resin batch quality control with stringent QC specifications to ensure lot-to-lot reproducibility, to ensure robust resin and column manufacturing processes, which is crucial in method development and data interpretation. Additionally, the use of polyether ether ketone (PEEK) for column hardware, recognized for its bioinert properties, minimizes nonspecific protein adsorption, contrasting with metal-based alternatives, and thus will make the column suitable for analyzing complex biological samples with high resolution and excellent reproducibility.

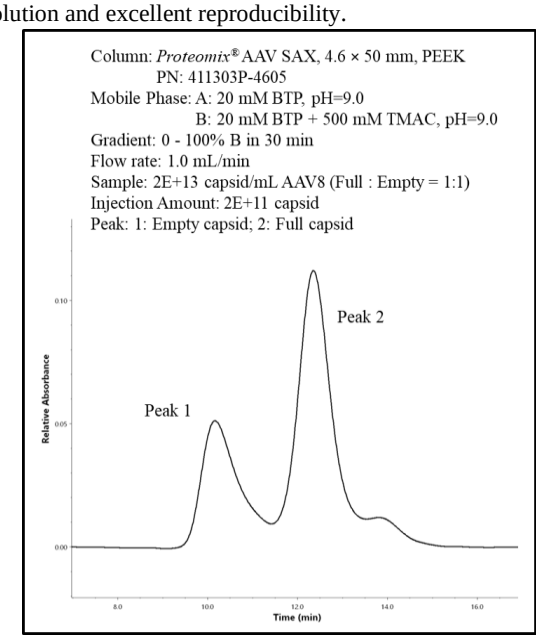


**Figure 2**. Separation of the empty and full capsid of AAV8 mixture on a 4.6 × 50 mm, *Proteomix*® AAV SAX column.

Typical column dimensions of *Proteomix*® AAV SAX, 4.6, I.D., and 50 mm length. Sepax also offers custom-made columns.

## Technical Specifications

| Phases | *Proteomix*® AAV SAX |
|---|---|
| Resin Matrix | Highly cross-linked monosized PS/DVB |
| Particle Size | 3 µm |
| Pore Structure | Non-porous |
| Functional Group | Quaternary ammonium |
| Dynamic Binding Capacity | ~ 36 mg/mL |
| pH Stability | 2 – 9, 9.5* |
| Operating Temperature Limit | 80 °C |
| Resin Pressure Limit | 8,000 psi |
| Column Operating Pressure Limit | 6,000 psi (a 4.6 x 50 mm PEEK column) |
| Typical Flow Rate | 0.1-1.0 mL/min (for a 4.6 x 50 mm PEEK column) |
| Mobile Phase Compatibility | Compatible with aqueous solution, a mixture of water and acetonitrile, acetone, or methanol. Typical buffers: phosphate, tris, acetate, and so on. |

*Extended use at higher pH levels (≥ 9.5) may reduce the column's lifetime.

## Safety Precaution

*Proteomix*® AAV SAX columns are normally operated under high pressure. Loose connections will cause leaking of buffers and injected samples, all of which should be considered hazards. In case of leaking, proper gloves should be worn for handling the leaked columns. When opening the columns, proper protection 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 installing the column to the system, first remove the end caps. Make the flow direction as marked on the column. Unless a user has a 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 the tubing firmly into the column end fitting. Slide the nut and ferrule forward, engage the threads, and finger-tighten the nut.

(c) While continuing to press the tube firmly into the end fitting, 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 0.2 µm filters before use. It is also strongly recommended to use a pre-column filter (≤ 0.5 µm frit) to protect the column. The *Proteomix*® AAV SAX columns are compatible with an aqueous mobile phase or a mixture of organic and water, such as methanol or acetonitrile and water. 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.

Noted that *Proteomix*® AAV SAX columns are compatible with nonionic and zwitterionic detergents. ***However, they are incompatible with anionic detergents.***

## Initial Column Preparation

Before the initial use of the Proteomix® AAV SAX column, it is crucial to perform a comprehensive column-level wash to ensure optimal performance and low noise levels during analytical operations. Follow the exemplified sequence with appropriate buffers and flow rates as outlined below:

| Column Wash with Tris Buffer (pH 8.0) | |
|---|---|
| Mobile Phase A | 20 mM TRIS Buffer, pH 8.0 |
| Mobile Phase B | 20 mM TRIS Buffer, pH 8.0 + 0.5 M NaCl |
| Flow Rate | 0.5 mL/min |
| Maximum Flow Gradient | 0.1 mL/min$^2$ |

| Wash Sequence | |
|---|---|
| **Mobile Phase** | **Operation Time** |
| Mobile Phase A | 1.0 hour |
| Mobile Phase B | 2.0 hours |
| Mobile Phase A | 4.0 hours or overnight |

By following this sequence, the column should exhibit very low noise levels, preparing the column for efficient analytical performance.

## Column Equilibration

This protocol helps to stabilize the column with the Bis-tris-propane (BTP) buffer system as an example, suitable for subsequent analytical uses. The flow rates and times can be adjusted based on specific experimental needs.

| BTP TMAC pH 9.0 Testing Condition | |
|---|---|
| Mobile Phase A | 20 mM BTP, pH=9.0 |
| Mobile Phase B | 20 mM BTP + 500 mM TMAC, pH=9.0 |
| Flow Rate | 0.5 - 1.0 mL/min |
| Maximum Flow Gradient | 0.1 mL/min$^2$ |

| Wash Sequence | | |
|---|---|---|
| **Mobile Phase** | **Operation Time** | **Flow Rate** |
| Mobile Phase B | 1.0 hour | 0.5 mL/min |
| Mobile Phase A | 0.5 hour | 0.5 mL/min |
| Mobile Phase A | until baseline (at least 1 hour for BTP system) | 1.0 mL/min |

## Post-Use Handling

To prolong the lifetime of the column, implement the following storage practice after use:

| Post-Use Wash with Tris Buffer (pH 8.0) | |
|---|---|
| Mobile Phase A | 20 mM BTP, pH=9.0 |
| Flow Rate | 0.5 mL/min |
| Maximum Flow Gradient | 0.1 mL/min$^2$ |
| Operation Time | 15 min |

Best practice for column storage to prolong the lifespan of the column: Use a third line on the system to wash the column to 20 mM TRIS Buffer (pH 8.0) after daily use. A volume of 5-10 column volumes (CV) is recommended for safe column maintenance. This routine washing helps to maintain the integrity and performance of the column, ensuring readiness for future analytical tasks.

## Column Care

***Shipping solvent*** New *Proteomix*® AAV SAX shipped in 20 mM Tris at pH 8.0.

***pH*** The optimum performance and operation for the longest lifetime are at pH 2 - 9. Extended use at a higher pH ≥ 9.5 will shorten the column lifetime. Prolonged exposure of the columns to high pH (≥ 9.5). conditions should be limited.

***Pressure*** Even though the non-porous *Proteomix*® AAV SAX resin can tolerate pressure up to 8,000 psi, the operating pressure of the PEEK hardware is normally under 6,000 psi. Continuous use at high pressure may eventually damage the column. Since the pressure is generated by the flow rate. The maximum flow rate is limited by the back pressure. It is expected that the backpressure might gradually increase with its service. A sudden increase in backpressure suggests that the column inlet frit might be plugged. It is recommended to wait until the pressure drops to zero to safely disconnect the column from the testing apparatus at the end of the test.

***Temperature*** The maximum operating temperature is 80 °C. The optimum temperature operation for the longest lifetime is 10 – 50 °C. Continuous use of the column at higher temperatures (> 80 °C) can damage the column, especially under extreme pH (> 12 or < 2.0).

***Flow rate range*** Normal operation is 0.1-1.0 mL/min for 4.6 mm I.D. columns but may vary for different operation systems. The maximum flow rate is limited by the pressure.

***Storage*** When not in use for an extended time, store the *Proteomix*® AAV SAX columns in 20 mM Tris at pH 8.0/0.01-0.05% $NaN_3$. Flush the column with the storage buffer for at least 5 column volumes. And then seal both ends with the removable end plugs provided with the column, to prevent the drying of the column bed.

## Column Protection

To extend the lifespan of the column, it is crucial to filter both the sample and mobile phases daily using 0.2 µm filters. Additionally, to protect against residual particulates from the sample or mobile phase, the use of a precolumn filter is recommended. Install a precolumn filter with a cutoff of ≤ 0.5 µm and replace it whenever an increase in back pressure or a decrease in column performance is observed. Following each cleaning or replacement of the precolumn filter, back flush the column with a high-salt solution (1 M NaCl) for 10 column volumes.

## Column Cleaning

(1) If a precolumn filter is used before the separation column, clean the precolumn filter first by flushing the filter in reverse flow direction using washing solutions for 15-30 min or replace the filter if the washing does not improve the column performance.

The washing solutions are 20-50 mM Tris pH 8 with 0.5 - 1 M chaotropic salts such as NaCl or KCl. Sometimes organic additives such as 5-15% ACN or IPA may help to clean out hydrophobic deposits.

(2) From time to time, some samples could get adsorbed onto the inlet frit or the packing material. When the adsorption accumulates to a certain level, it is usually indicated by the backpressure being increased and the peak becoming broader. When this occurs, it is time to clean your column. The general guidelines for column cleaning are the following:

1. Disconnect the column from the detector and directly connect the column to the waste.
2. Run the column at less than 50% of the maximum recommended flow rate. Monitor the back pressure. If you see the pressure is much higher than the normal operating conditions, you need to lower the flow rate or change the washing buffer as the cleaning solutions may be of different viscosities.
3. Typically, 10-15 column volumes of cleaning solution are adequate. General guidelines for selecting cleaning solutions include using a low-pH salt solution to remove basic proteins, a high-pH salt solution for acidic proteins, and organics for hydrophobic proteins.

## Order Information

| Part Number | Particle Size | Pore Size | ID × Length | Hardware |
|---|---|---|---|---|
| **411303P-4605** | 3 µm | Non-Porous | 4.6 × 50 mm | PEEK |
| **411303P-4610** | 3 µm | Non-Porous | 4.6 × 100 mm | PEEK |