Sepax Technologies, Inc.

Delaware Technology Park
5 Innovation Way, Newark, DE 19711, USA
Tel: (302) 366-1101; Fax: (302) 366-1151
www.sepax-tech.com

ProAqa Affinity Column Manual

Column Information

ProAqa affinity columns are high-flow and high-pressure columns that are designed for operation on HPLC and FPLC systems. The average 20 µm packing support is composed of porous poly(styrenedivinylbenzene) (PS/DVB) bead with a hydrophilic coating grafted on the surface. On the top of the coating, recombinant protein A is attached via chemical bonding, which binds Fc-containing immunoglobulin proteins except IgG3. The columns can be used for the quantification and small-scale purification of antibodies and fusion proteins binding specifically to the recombinant protein A.

Dynamic Binding Capacity

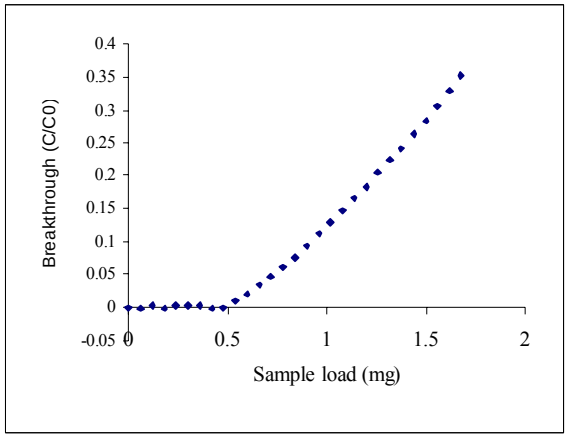

Figure 1. Breakthrough curve of antibody sample (Mab 321 from Sepax Technologies, Inc.) for a ProAqa 2.1×30 mm column. Mobile phase: sodium phosphate buffer, 0.1M, pH 7.4, NaCl, 0.15M, 0.15mg/mL Mab 321; Flow rate: 2.0 mL/min; Detection wavelength: 280nm

Technical Specifications

Item	Details
Support matrix	Cross-linked poly(styrenedivinylbenzene)
Immobilized ligand	Recombinant protein A
Dynamic binding capacity	9.3 mg/ml (DBC curve can be seen in Fig. 1 with Mab 321 sample.
Shipping solution	0.1 M sodium phosphate, pH7, 0.02% sodium azide
Pressure limit	180 bar
Maximum operating flow rate	5000 cm/hour
pH range	2-10
Ionic strength	0-5 M, all common salts
Buffer	Common buffers, including 4 M urea, 3 M guanidine hydrochloride, ethylene glycol, and detergents.
Solvent	Water, 0–90% ethanol, acetonitrile and common organic solvents. *NOTE: Do not expose columns to any media which could degrade and destroy Protein A.*
Operating temperature	2-40 °C, **DO NOT FREEZE**

Column Installation and Operation

When column is shipped or not in use, it should be 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 or FPLC 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. The fitting of the column is an 10-32 female fitting. Do not use any fitting that requires tightening with a wrench. Over-tightening can strip the threads of the column.

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

Before you use the column for the first time, pump 5-10 column volumes (CVs) of elution buffer to remove the shipping solvent. Equilibrate with 10 to 15 CVs of starting/wash buffer. Always use a pre-column filter (0.5 µm) to minimize column fouling (Part No. 102000-P356, 102001-P356).

Blank Run

Always use buffers of the highest purity and degas and filter (0.22 or 0.45 µm) all buffers prior to use.

Before loading sample, always execute blank runs and examine peak integration results carefully for artifact or an improperly drawn baseline. To minimize the baseline changes between binding and elution buffer changes. It is recommended to use:

(a) 50 mM phosphate pH 7, 0.15 M NaCl as the starting/wash buffer, and

(b) 0.1% (12 mM) HCl 0.15 M NaCl as the elution buffer.

This is the most effective wash/eluent system with the minimum RI shift. Hydrochloric acid (HCl) can denature antibodies, so it

is not recommended when biological activity is required in the eluting product.

Starting/Wash Buffer

(a) In most cases, simple buffers such as 10 to 50 mM phosphate or Tris can be used.

(b) The starting/wash buffer pH can range from 6.0 to 9.0, but note that binding is usually strongest in the higher pH range.

(c) Add some salt (0.1 to 0.2 M NaCl or KCl) to prevent nonspecific adsorption due to protein/protein interactions.

Elution Conditions

For analytical applications, use 25-100 mM phosphate, pH 2.0-3.5, without or with sodium chloride up to 150 mM. Other elution buffer components that may be used include hydrochloric acid, glycine, citrate, acetate, or other components that buffer well at low pH.

For preparative applications, the following conditions can be used.

(a) To elute most antibodies, reduce the pH to 2-3, common buffer systems include phosphate, acetate, hydrochloric acid, and glycine. Buffer concentrations can range from 6-100 mM or 2-20% (v/v), depending on buffer system.

(b) 6-12 mM HCl with 0.15 M NaCl can be used to obtain the desired pH range and minimizes the refractive index effect.

(c) Because antibodies differ by both species and subclass in their binding/elution behavior, the best elution condition should be determined empirically.

Sample Preparation and Sample Load

To ensure efficient binding and prevent column-frit fouling, samples are typically prepared as following:

(a) Dissolve or exchange samples into the starting/wash buffer. This is particularly important for large samples (greater than 25% of the column volume).

(b) Centrifuge or filter samples (0.22 or 0.45 µm) before injection.

(c) Heat-treat serum samples (56 °C for 30 minutes) to remove residual fibrinogen that can clog the column on multiple runs.

(d) Delipidate samples, if possible. Lipids can cause irreversible fouling.

(e) All samples should be filtered through 0.45 µm or 0.2 µm filters prior to use.

To ensure efficient binding and prevent resin and column fouling, sample load need to be determined:

(a) The example of ProAqa dynamic binding capacity is listed in Table 1.

(b) The binding capacity for other antibodies depends on the antibody source and subclass and the ligand used, but it is generally lower than the capacity for IgG listed in Table 1.

(c) In analytical applications, minimum and maximum load is determined by the linearity of the standard curve.

Column Protection

In addition to filtering the mobile phase and the sample, always install a pre-column filter in front of the column. In most cases, a pre-column filter helps to remove the residual particulates in the sample or the mobile phase, or leached from HPLC or FPLC system, such as pump and injector seals.

Column Clean-up and Regeneration

Columns are generally robust. If you reuse the column, monitor column backpressure and run an assay control sample. If backpressure increase or control sample recovery changes, clean the column to remove residual material from the column frits and from the resin. Typical cleaning solutions include 2-6 M guanidine hydrochloride, 1 M acetic acid, 20% ethanol, 1 M acetic acid plus 20% ethanol, 20% isopropanol, elution buffer titrated to pH1.5-2.0, and elution buffer plus 1-2 M sodium chloride.

To clean, make 2 or 3 injections of cleaning solution at a volume equal to the column bed volume, followed by 2 or 3 injection of equilibration buffer- for example, 2×100 µL cleaning solution, 2×100 µL equilibration buffer. Alternatively, you can run multiple column volumes of cleaning solution. You can reverse flow to help clean the top frit and then return the normal cleaning sequence.

Storage

When not in use for extended time, store the ProAqa columns under the following conditions:

(a) In a neutral pH solution with a bacteriostatic agent such as 0.02% sodium azide.

(b) In the refrigerator, but **DO NOT FREEZE THEM**!

(c) With the endcaps in place, carefully sealed to prevent drying. Drying results in decreased chromatographic efficiency.

Safety Precaution

ProAqa 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.

Support

For service and technical support, go to www.sepax-tech.com, or call Tool-free in US: (877)-SEPAX-US.

Order Information

Product Description	Dimension (ID×Length) (mm)	Column Volume (mL)	P/N#
ProAqa (stainless steel)	2.1x30	0.1	512001-2103
ProAqa (PEEK)	4.6x50	0.8	512001P-4605
	4.6x100	1.7	512001P-4610
	10x100	7.9	512001P-10010