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ProAqa Excel Affinity Column Manual

Column Information

Sepax ProAqa Excel affinity column is designed for fast IgG titer determination. The base resin is composed of highly crossed linked porous poly(styrene/divinylbenzene) (PS/DVB). The monosized 20 μm beads have a very narrow size distribution of D_{90}/D_{10} <1.3 with a pore distribution between 1000-2000 Å. The porous resin surface was engineered by grafting a hydrophilic coating. On the top of the coating, a recombinant protein A is attached via chemical bonding, which binds Fc-containing immunoglobulin proteins except IgG3.

The resin is mechanically robust and can tolerate high pressure operation. It can be applied for the operation on UPLC, HPLC and FPLC systems at high flow rate. Routine below 2 min assay allows robust high through-put screen of large quantity samples at a short period time. A shorter time analysis < 2 min such as 0.5 min is achievable as long as the back pressure does not exceed the maximum limit.

A typical chromatogram is shown in **Figure 1**. The columns can be used for the quantification of antibodies and Protein A binding fusion protein, up to a titer of 7.5 mg/mL at 280 nm and up to 40 mg/mL at 300 nm as detection wavelength **Figure 2** and **Figure 3**.

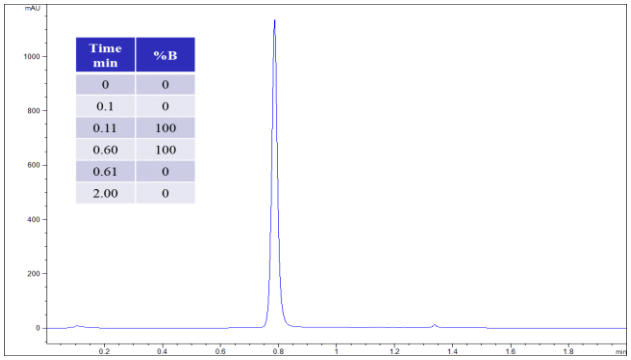

Figure 1. Typical chromatogram of hIgG analysis on a ProAqa Excel 2.1×30 mm column. Mobile phase: A: 50 mM sodium phosphate, 150mM NaCl, pH 7.0, B: 100 mM Glycine, pH 2.5; Column temperature: Ambient; Flow rate: 1.0 mL/min; Detector: 280 nm; Sample: 1.875 mg/mL Human IgG in water; Injection amount: 10 μL.

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 finger tighten 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).

Technical Specification

Item	Details
Support Matrix	20 μm, Poly(styrene/divinylbenzene)
Pore Size	1000-2000 Å
Immobilized ligand	Recombinant Protein A
Ligand loading	>5 mg/mL resin
Shipping condition	0.1 M sodium phosphate, pH 7.0, 150 mM NaCl, Temperature 2-8 °C
Pressure limit	200 bars
Maximum operating flow rate	8600 cm/hour
pH range	1.2-13.0
Ionic strength	0-5 M, all common salts
Running Buffer	Common buffers, including phosphate, acetate, 4 M urea, 3 M guanidine hydrochloride, ethylene glycol, and detergents.
Storage solvent	Water, 0-20% ethanol, acetonitrile, and common organic solvents. *NOTE: Do not expose columns to any media which could degrade and destroy Protein A.*
Operating temperature	10-35 °C, **DO NOT FREEZE**
Column lifetime	2000 injections at optimum operating conditions
CIP	2-6 M guanidine hydrochloride, 1 M acetic acid, 20% ethanol, isopropanol, 0.1 M NaOH

Blank Run

Always use buffers of the highest purity. Degas and filter (0.22 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, 0.15 M NaCl as the starting/wash buffer, and

(b) 100 mM Sodium phosphate, 0.15 M NaCl, pH 2.5, or 100 mM Glycine, 0.15 M NaCl, pH 2.5 as the elution buffer.

These are the most effective wash/eluent system with the minimum signal artifacts. 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 100 mM phosphate or Tris can be used.

(b) The binding /wash buffer pH can range from 6.0 to 7.5, but please 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 sodium phosphate, hydrochloric acid, glycine, citrate, acetate, or other components that buffer well at low pH.

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 with 0.22 µm membrane 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.

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

(a) An example of ProAqa Excel dynamic testing range is shown in **Figure 2** and **Figure 3**.

(b) The binding capacity for other antibodies depends on the antibody source and subclass.

(c)The binding and elution condition need to be optimized and confirmed with samples to make sure the enough binding equilibration and complete elution are achieved

(d) In analytical applications, minimum and maximum load should be determined by the linearity of the standard curve. (as shown in **Figure 2**. For a typical titer analysis with a target concentration range 0.029 - 7.500 mg/mL, detection wavelength can be set at 280 nm. If a very high titer analysis (up to 40 mg/mL) is needed, the wavelength can be set at 300 nm as shown **Figure 3**. In this application, dual wavelength detection is recommended (at 280 and 300 nm).

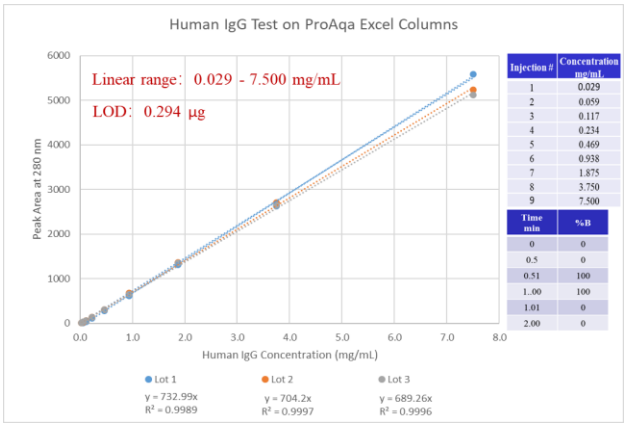

Figure 2. Linear range and Lot to Lot Consistency Tests of ProAqa Excel 2103 column monitored at UV 280 nm. Mobile phase: A: 50 mM Phosphate, 150mM NaCl, pH 7.0, B: 100 mM Glycine, pH 2.5; Column temperature: ambient; Flow rate: 1.0 mL/min; Sample: Human IgG in water as shown in table; Injection amount: 10 µL. Buffer changes are shown in the table.

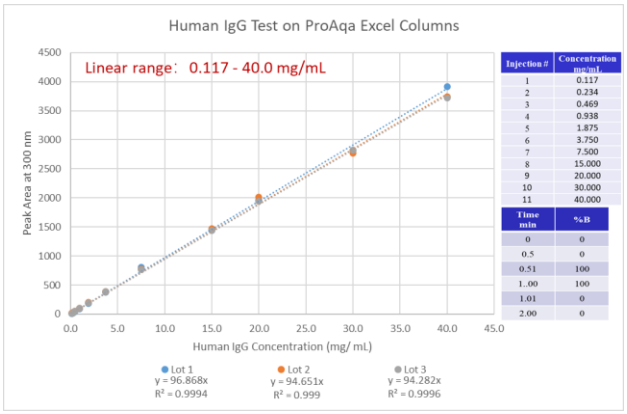

Figure 3. Linear range and Lot to Lot Consistency Tests of ProAqa Excel 2103 column monitored at UV 300 nm. Mobile phase: A: 50 mM Phosphate, 150mM NaCl, pH 7.0, B: 100 mM Glycine, pH 2.5; Column temperature: ambient; Flow rate: 1.0 mL/min; Sample: Human IgG in water as shown in table; Injection amount: 10 µL. Buffer changes are shown in the table.

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 residual 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, isopropanol, elution buffer plus 1-2 M sodium chloride and 0.1 M NaOH.

To clean, wash the column with cleaning solution at a volume equal to 15 x column bed volume at a flow rate of 0.1 -0.2 mL/min, followed by washing with equilibration buffer. 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 Excel 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 2-8 ºC, 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 Excel 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 fine polymer particles.

Support

For service and technical support, go to www.sepax-tech.com, call Toll-free in US: (877)-SEPAX-US, or email us at techsupport@sepax-tech.com.

Order Information

Standard Size

Product Description	Dimension (ID×Length) (mm)	Column Volume (mL)	P/N#
ProAqa Excel	2.1 x 30 PEEK	0.10	271120980-2103P
	2.1 x 30 Stainless Steel	0.10	271120980-2103S

Custom Size

Product Description	Dimension (ID×Length) (mm)	Column Volume (mL)	P/N#
ProAqa Excel	2.1 x 50 PEEK	0.17	271120980-2105P
	2.1 x 50 Stainless Steel	0.17	271120980-2105S
	4.6 x 35 Stainless Steel	0.58	271120980-4603S
	4.6 x 50 PEEK	0.83	271120980-4605P
	4.6 x 50 Stainless Steel	0.83	271120980-4605S
	4.6 x 100 PEEK	1.66	271120980-4610P
	4.6 x 100 Stainless Steel	1.66	271120980-4610S