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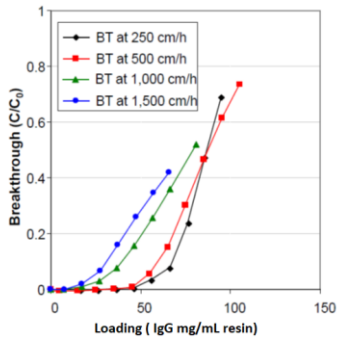
Protein A-Sil Column User Manual

Column Information

Protein A-Sil affinity columns are designed for application on U/HPLC and FPLC systems, which can tolerate high-flow and high-pressure. The Protein A-Sil packing is made of porous silica (30 µm, 1000 Å) with recombinant protein A chemically conjugated via hydrophilic coating.

The Protein A-Sil columns can be applied to the titer determination and small-scale purification of antibodies, Fc containing fusion proteins binding specifically to the recombinant protein A.

Dynamic Binding Capacity



Linear Velocity	DBC at 10% BT
cm/h	mg/mL
250	67.4
500	59.0
1,000	39.7
1,500	31.1

Operational conditions:
- Column: 5 mm i.d.; 100 mm L
- V_{col} = 1.96 mL
- [hIgG] = 3.2 g/L
- Fractions are collected and analyzed by HPLC to determine Ab content.

Figure 1. Breakthrough curve of antibody sample (hIgG)

Technical Specifications

Support matrix	Porous silica
Immobilized ligand	Recombinant protein A
Dynamic binding capacity	67 mg/ml at flow rate 250 cm/h (DBC curve can be seen in Fig. 1 with a hIgG)
Shipping solution	0.1 M sodium phosphate, pH7
Pressure limit	5000 PSI*
Maximum operating flow rate	1500 cm/hour
pH range	2-13
Operating temperature	2-40 °C, **DO NOT FREEZE**

Antibody Titer Determination

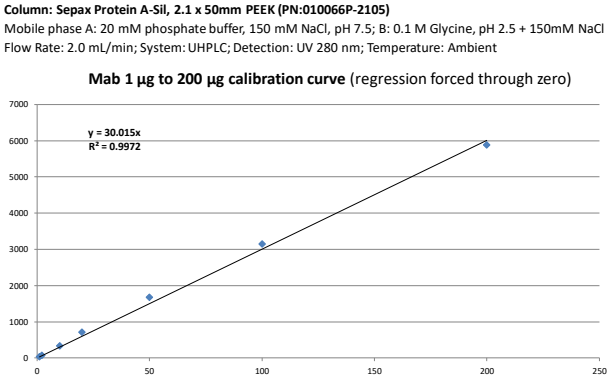


Figure 2. Purified Mab Loading Calibration Curve

Column Installation and Operation

When column is shipped or not in use, it should be 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 special purpose to reverse the flow direction, for example, removal of the inlet plugging, 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 U/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).

Blank Run

Always use buffers of the highest purity practical 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) 20 mM phosphate buffer, 150 mM NaCl, pH 7.5 as the starting/wash buffer, and

(b) 0.1 M Glycine, pH 2.5 + 150mM 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 Glycine, pH 2.0-3.5, without or with sodium chloride up to 150 mM. Other elution buffer components that may be used including phosphate, 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.

(d) Do not expose columns to any media which could degrade and destroy Protein A.

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 Protein A-Sil dynamic binding capacity is listed in Figure 1.

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

(c) In analytical applications, minimum and maximum load is determined by the linearity of the standard curve as Shown in Figure 2.

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 U/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 the resin. General cleaning solutions include 0.1M NaOH/0.5M NaCl or PAB (120 mM H4PO4, 167 mM Acetic acid, 2.2% Benzyl Acohol). PAB is a gentler cleaning solution than NaOH.

Extended use of NaOH solution will damage the column resin. CIP can be achieved using 2 CV of CIP solution with 0.1 mL/min flow rate.

Storage

When not in use for extended time, store the Protein A-Sil columns under the following conditions:

(a) In a neutral pH solution with a bacteriostatic agent

(b) 2-8 °C, **DO NOT FREEZE**!

(c) Endcaps in place to prevent column drying. Drying results in decreased chromatographic efficiency.

Safety Precaution

Protein A-Sil columns are normally operated under pressure. Loose connections will cause leaking of buffers and injected samples, all of which should be considered as chemical hazards. In the case of leaking, proper gloves should be worn for handling the leaking columns. When opening the columns, proper protections should be used to avoid inhalation of small polymer particles.

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