

ProAqa Excel Protein A Affinity HPLC Column

Sepax Technologies, Inc.

FOR FAST ANTIBODY TITER DETERMINATION

ProAqa Excel Protein A Affinity HPLC columns are specifically designed for **fast, accurate and robust** titer determination and quantification of monoclonal antibodies during cell line selection or upstream bioprocess optimization and quality control.

The base resin is composed of monosized 20 μm , 1000-2000 \AA , highly crossed linked PS/DVB beads coupled with a recombinant Protein A ligand which binds Fc-containing immunoglobulin protein, except IgG3. The resin is mechanically robust and can tolerate high-flow and high-pressure operation.

Technical Description

ProAqa Excel	Details
Support Matrix (Particle size, Pore Size)	Monosized PS/DVB (20 μm , 1000 - 2000 \AA)
Immobilized Ligand	Recombinant Protein A
Column size ID x L	2.1 mm x 30 mm
Column Format	PEEK or Stainless Steel
Pressure Limit	200 bar (Pressure Drop)
pH Range	1.2 - 13.0
Maximum Flow Rate	5 mL/min
Recommended Flow Rate	1 - 3 mL/min
CIP	0.1 M NaOH
Binding Buffer pH	6.6-7.5
Lifetime	>2000
Standard Injection	10 μL
IgG Testing Concentration	0.029 - 40 mg/mL*
LOD	0.29 μg

(*UV 280 nm: 0.029 - 7.500 mg/mL, UV 300 nm: 0.117-40.000 mg/mL)

Features:

Rapid Antibody Titer Determination:

- Up to 3 mL/min
- < 0.5 minutes total cycle time, including equilibration

Wide linear response range up to 40 mg/mL:

- UV 280 nm: 0.029-7.50 mg/mL, LOD 0.294 μg
- UV 300 nm: 0.117-40.0 mg/mL

Robust: > 2000 Injection Lifetime

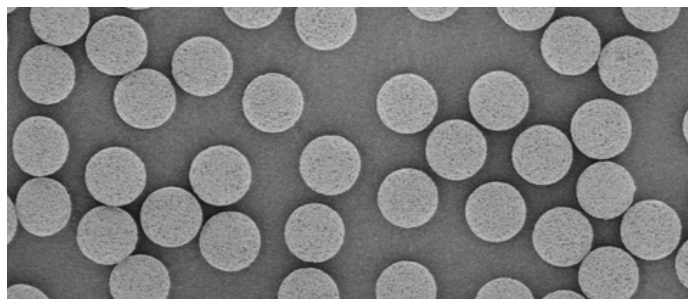
Reproducible: High Lot to Lot Consistency

Applicable: Various amounts of CHO cell proteins

Compatible: UPLC, HPLC and FPLC

ProAqa Excel Base Resin SEM

Monosized beads (20 μm) with D90/D10<1.3

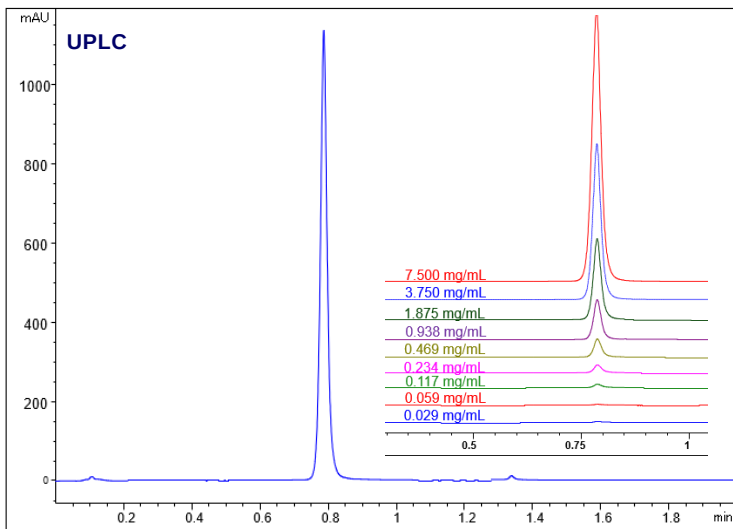


Experimental Conditions

System:	UPLC Agilent 1290; HPLC Agilent 1260
Column:	20 μm , 2.1 x 30 mm; 271120980-2103S
Binding Buffer:	50 mM Phosphate, 150mM NaCl, pH 7.0
Elution Buffer:	100 mM Glycine, pH 2.5
Gradient:	0.0 - 0.10 min: binding buffer 0.11 - 0.60 min: elution buffer 0.60 - 2.00 min: binding buffer
Flow Rate:	1.0 mL/min
Human IgG Concentration:	0.029 to 30 mg/mL
Sample:	2.0 mg/mL hIgG + lysate
Column Temperature:	23 $^{\circ}\text{C}$
Injection Amount:	10 μL
Detection:	UPLC - UV @ 280 nm HPLC - UV @ 280 nm and 300 nm

Figure 1. Human IgG Test - Linear Range and LOD

The ProAqa Excel column can be used for the quantification of antibodies and Protein A binding fusion proteins. A typical Human IgG (hIgG) test chromatogram is shown in Figure 1.



Wide linear response range has been achieved, up to a titer of 7.5 mg/mL at 280 nm with LOD 0.294 µg, and up to 40 mg/mL at 300 nm as detection wavelength as shown in Figure 2-3.

Figure 2-3. Linear Range and Lot to Lot Consistency

ProAqa Excel columns show great reproducibility through lot to lot consistency tests, on both UV 280 and 300 nm.

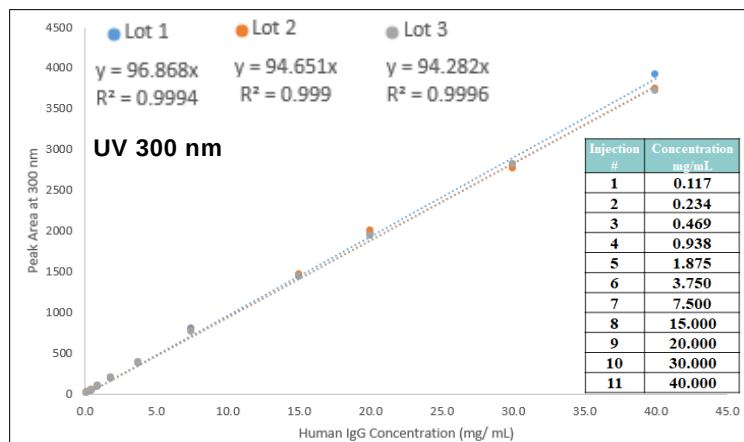
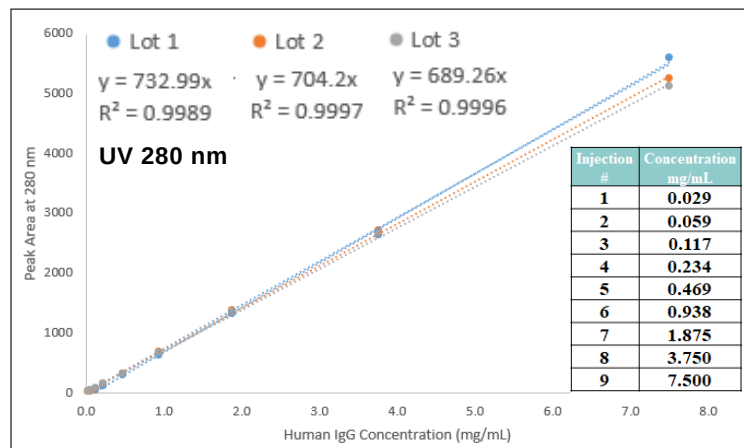
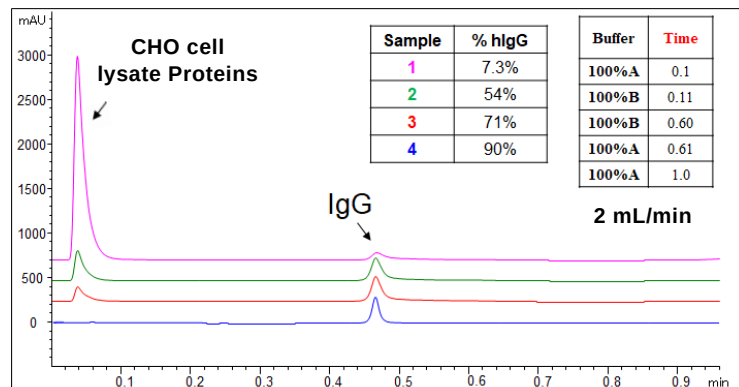


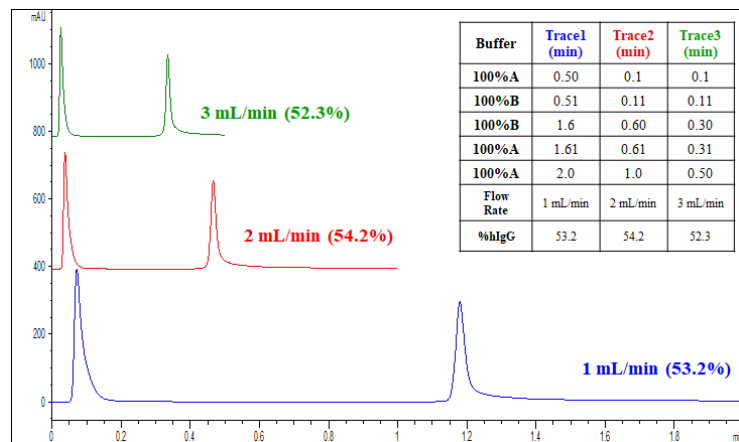
Figure 4. Various Amounts of CHO Cell Lysate Proteins

The ProAqa Excel column offers excellent performance at different levels of CHO cell lysate protein concentration, 7.3%, 54%, 71% and 90% in this study, respectively.



ProAqa Excel columns can tolerate high flow rate and high-pressure operation. Flow rates from 1 mL/min to 3 mL/min were carried out in the hIgG test with CHO Cell Lysate Proteins. Less than 0.5 minutes total cycle time was achieved at 3 mL/min, and no significant change in the % of hIgG was observed.

Figure 5. Fast hIgG Test with CHO Cell Lysate Proteins



Fast Titer Determination Analysis can be achieved by optimizing the running conditions, including flow rate and equilibration. Different flow rates from 1 - 5 mL/min were studied as shown in Figure 6. Faster flow will reduce the total assay time, but also result in lower signal and sensitivity.

Figure 6. Fast Assay Study - Flow Rate & Equilibration

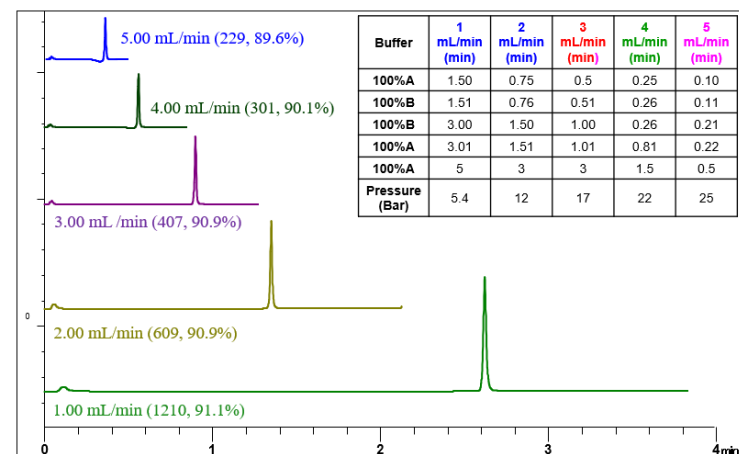


Figure 7. Column Life Time Test

ProAqa Excel columns are very robust and can last more than 2000 injections with minimal performance changes in retention time, peak area or symmetry. A CIP wash with 0.1 M NaOH for 15 minutes was performed after 1700 injections in below study as shown in Figure 7.

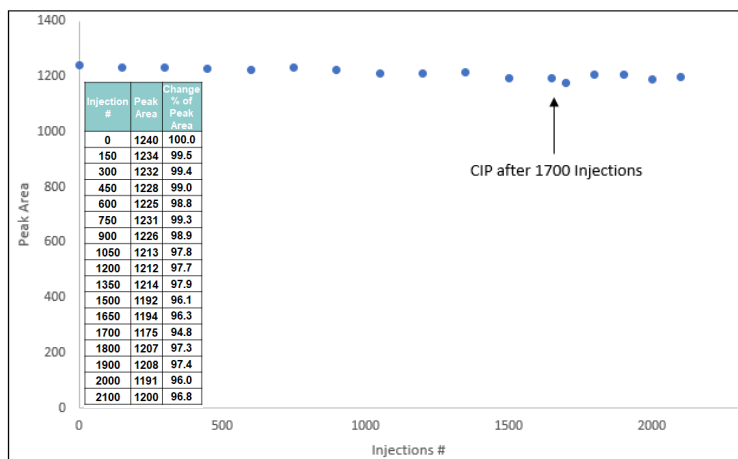
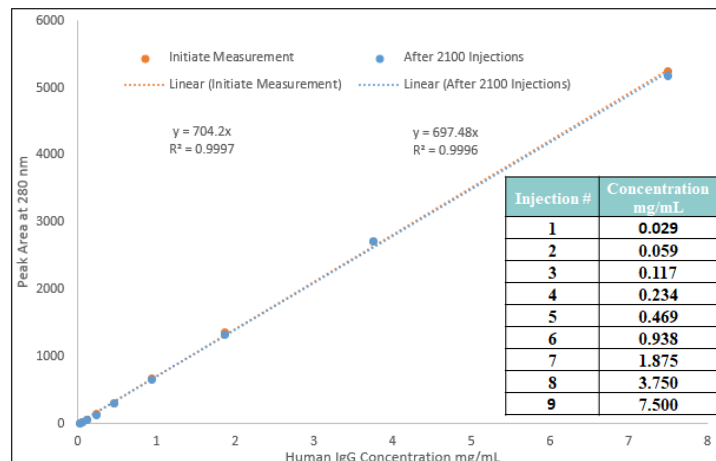


Figure 8. Durability and Linear Range Test after CIP

A Linear range test was performed on the ProAqa Excel column after 2000 injections. No significant change in the calibration curve for Human IgG was observed. The column still maintained its high loading capacity with an excellent linearity.



Ordering Information

Sepax ProAqa Excel Columns	
Part Number	Size
<i>Standard</i>	
271120980-2103P	2.1 x 30 PEEK
271120980-2103S	2.1 x 30 Stainless Steel
<i>Custom</i>	
271120980-2105P	2.1 x 50 PEEK
271120980-2105S	2.1 x 50 Stainless Steel
271120980-4603S	4.6 x 35 Stainless Steel
271120980-4605P	4.6 x 50 PEEK
271120980-4605S	4.6 x 50 Stainless Steel
271120980-4610P	4.6 x 100 PEEK
271120980-4610S	4.6 x 100 Stainless Steel

Sepax Affinity Product Family

- [ProAqa Excel Protein A Affinity Column](#)
- [MabPurix Protein A Affinity Resin](#)
- [Monomix dT20 Affinity Resin](#)
- [Monomix MC-Aminodiol Affinity Resin](#)
- [Monomix Affinity HSA Resin](#)
- [Polar MC-IMAC Excel Affinity Resin](#)
- [Bioservice Affinity HSA Column](#)
- [Custom Affinity Service](#)

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