High Throughput and Robust Method of Analysis of a Variety of Immunoglobulin G (IgGs) Using an Analytical Recombinant Protein A Affinity Chromatography Column

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Introduction

- IgG titer analyses, using protein A chromatography, are routinely performed in early phase development in order to select stable clones that express the highest concentration of antibody.
- While commercially available columns derived from native protein A have reasonable binding capacity for human IgG_1, IgG_2 and IgG_4, they possess no or limited binding affinity for IgG subclasses produced by different host cell sources (Figure 1).
- In this study, a TSKgel® Protein A-5PW column, containing a recombinant protein A ligand, comprised of a code-modified hexamer of the C domain (Figure 2), is shown to exhibit enhanced binding to various IgG subclasses and subtypes.

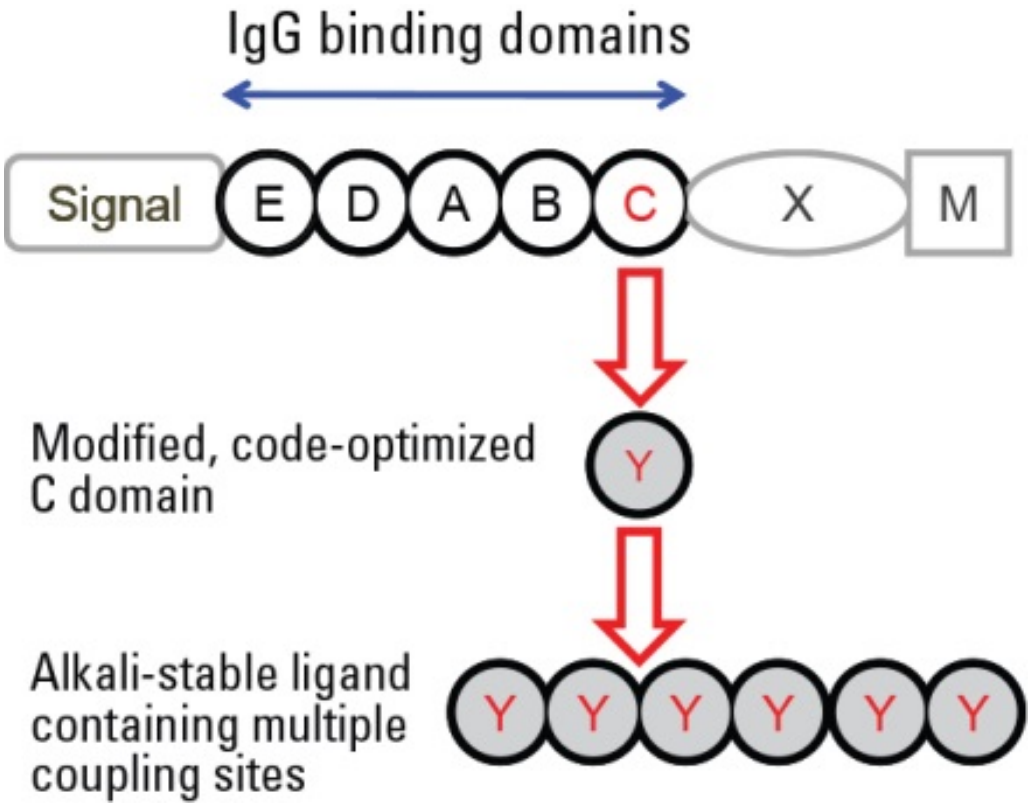
Introduction

Figure 1: The Affinity of TSKgel Protein A-5PW Columns for Various Species of Immunoglobulin Subtypes

Species	Subclass	Protein A ligand of Protein A-5PW	Native Protein A
Human	IgG_1 IgG_2 IgG_3 IgG_4	+++++ +++++ - +++++	++++ ++++ - ++++
Mouse	IgG_1 IgG_{2a} IgG_{2b} IgG_3	++++ +++++ +++++ ++++	+ ++++ +++ ++
Rat	IgG_1 IgG_{2a} IgG_{2b} IgG_{2c}	++++ - +++ ++++	- - - -
Goat	IgG_s	++++	-
Chicken	IgY	-	-
Rabbit	IgG	+++++	++++

Introduction

Figure 2: Ligand Structure of TSKgel Protein A-5PW Columns



Conditions and Materials

Columns: TSKgel Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm
Competitor Protein A column, 20 µm, 2.1 mm ID × 3.0 cm

Binding buffer: 20 mmol/L sodium phosphate, pH 7.4

Elution buffer: 12 mmol/L HCl

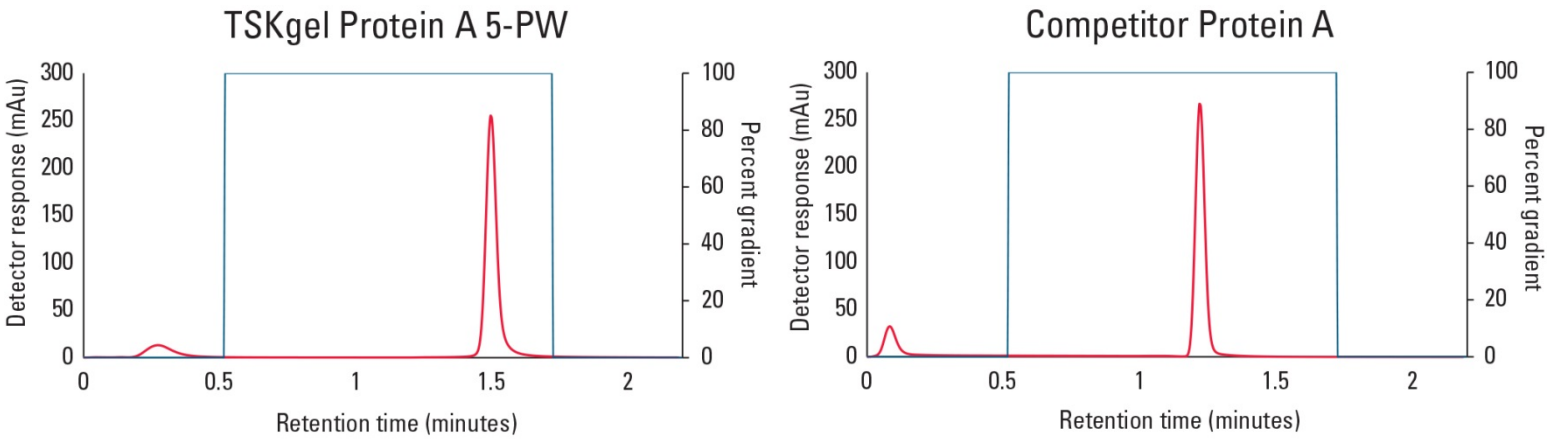
Step gradient: 0.0 - 0.5 min: binding buffer
0.51 - 1.7 min: elution buffer
1.71 - 2.2 min: binding buffer

Flow rate: 2 mL/min

Detection: UV @ 280 nm

	Conc. (mg/mL)	Injection vol.
Mouse IgG_1	3 mg/mL	5 µL
Rabbit IgG	1 mg/mL	5 µL
Goat IgG	1.6 mg/mL	5 µL

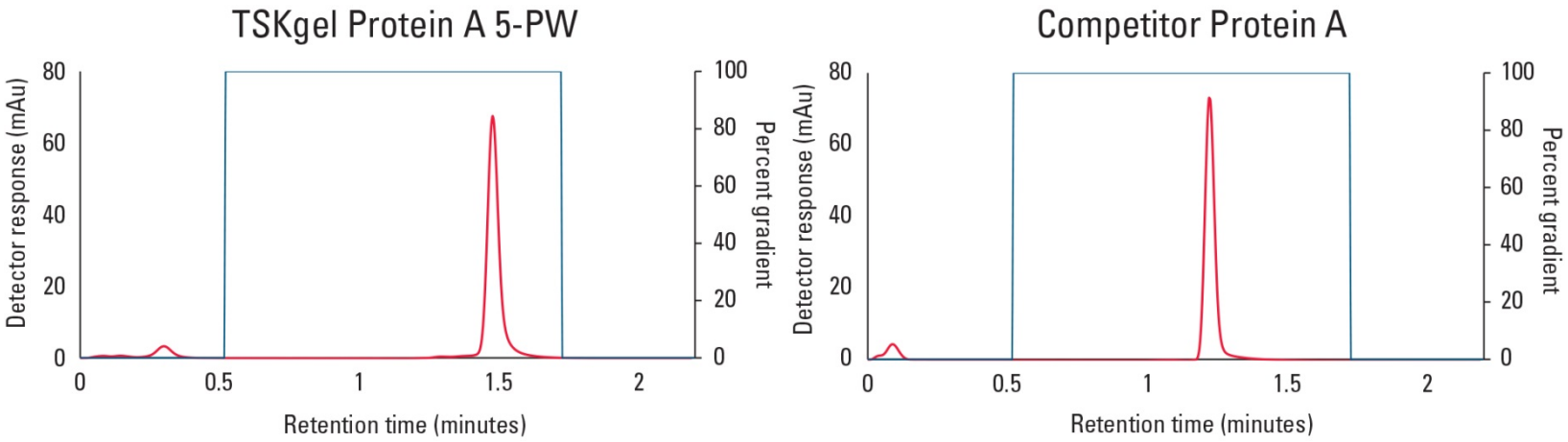
Affinity for Mouse IgG_1



Column	Total Area (mAu*min)	Unbound Area (mAu*min)	Unbound Relative Area, %	IgG Area (mAu*min)	IgG Relative Area, %
TSKgel Protein A 5-PW	864	98	11.3%	766	88.7%
Competitor Protein A	791	122	15.4%	669	84.6%

- **Both columns exhibit similar affinity for Mouse IgG_1.**
- **Based on the results of the chromatograms, it is suggested that the sample contains a small percentage of impurity.**

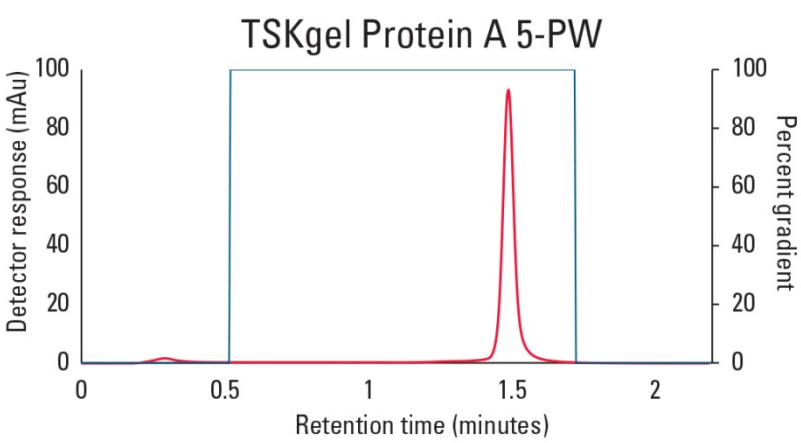
Affinity for Rabbit IgG

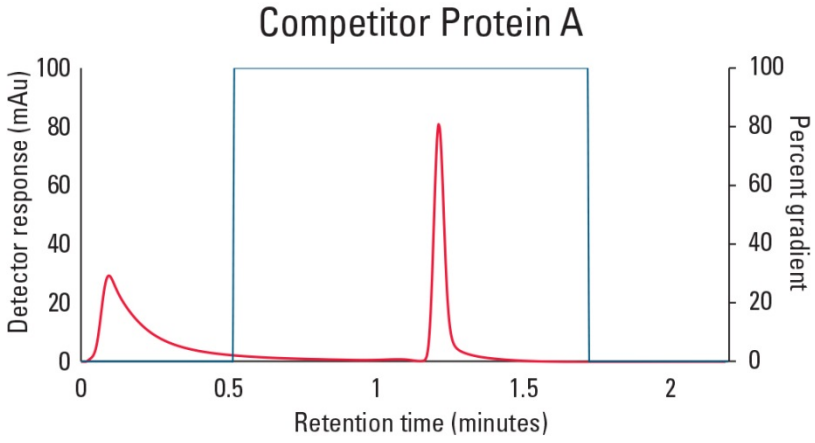


Column	Total Area (mAu*min)	Unbound Area (mAu*min)	Unbound Relative Area, %	IgG Area (mAu*min)	IgG Relative Area, %
TSKgel Protein A 5-PW	198	14	7.1%	184	92.9%
Competitor Protein A	209	17	8.3%	190	91.7%

- **Both columns exhibit similar affinity for Rabbit IgG.**
- **Based on the results of the chromatograms, it is suggested that the sample contains a small percentage of impurity.**

Affinity for Goat IgG

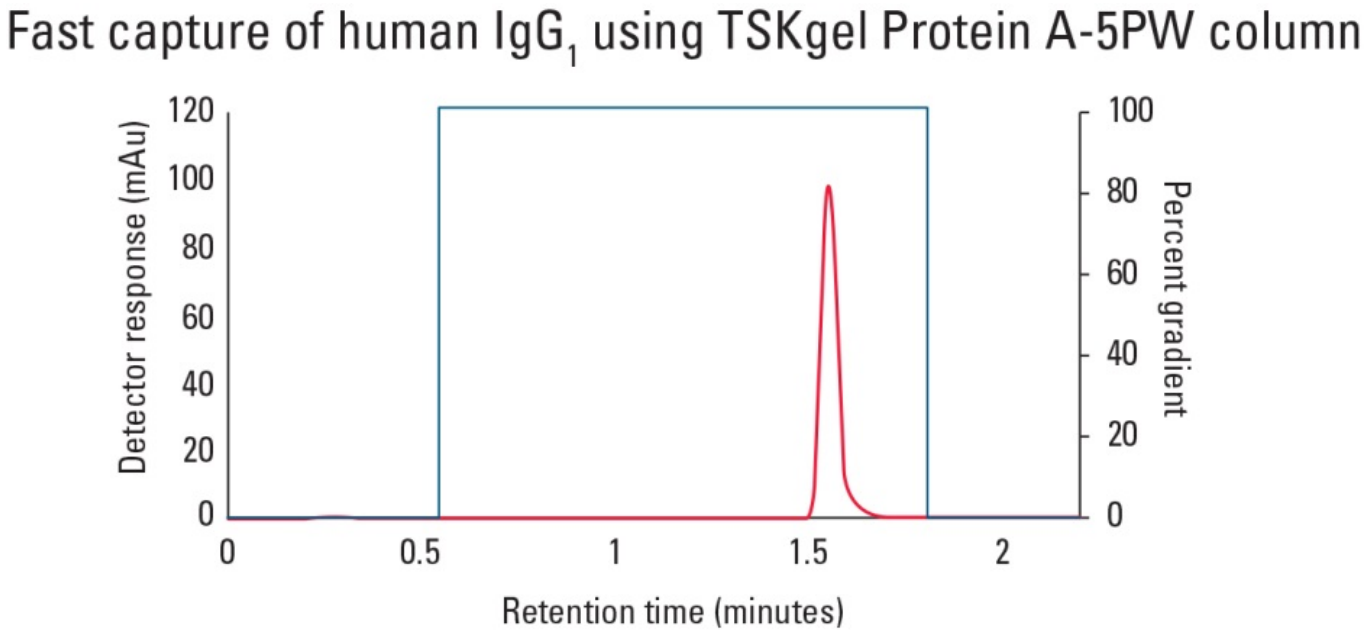




Column	Total Area (mAu*min)	Unbound Area (mAu*min)	Unbound Relative Area, %	IgG Area (mAu*min)	IgG Relative Area, %
TSKgel Protein A 5-PW	303	15	4.9%	288	95.1%
Competitor Protein A	358	211	58.9%	147	41.1%

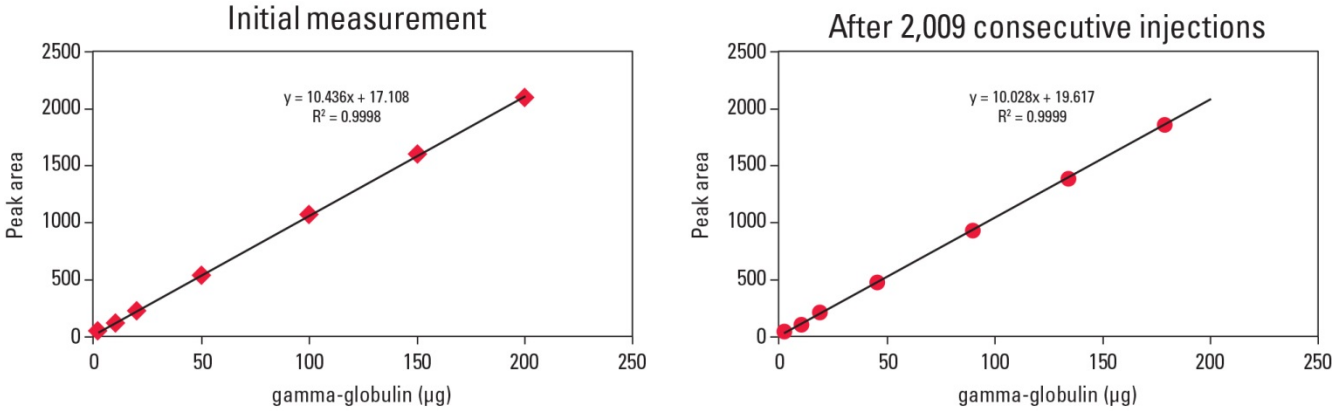
The TSKgel Protein A-5PW column demonstrates greater affinity for Goat IgG.

Affinity for Human IgG_1



- **Human IgG_1 was injected onto a TSKgel Protein A-5PW column for titer analysis.**
- **The run was completed in less than 2.2 minutes, including bind, wash, and re-equilibration steps.**
- **The peaks were eluted within the elution step of the chromatographic conditions as shown in the figure.**

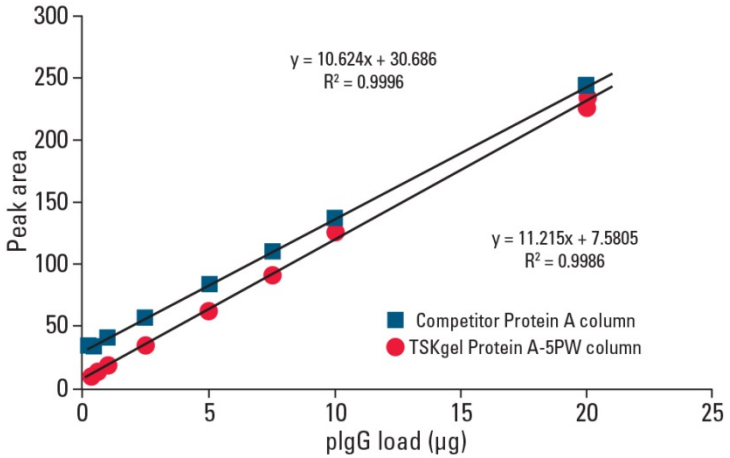
Linearity Analysis with TSKgel Protein A-5PW Column



Column:	TSKgel Protein A-5PW, 4.6 mm ID × 3.5 cm
Binding buffer:	20 mmol/L sodium phosphate buffer, pH 7.4
Elution buffer:	20 mmol/L sodium phosphate buffer, pH 2.5
Stepwise gradient:	0 - 0.5 min: binding buffer 0.5 - 1.1 min: elution buffer 1.1 - 2.0 min: binding buffer
Flow rate:	2 mL/min
Detection:	UV @ 280 nm
Sample:	polyclonal IgG (0.1 – 10 g/L), 20 µL (2 - 200 µg)

- **Determination of mAb concentration requires a column with excellent linearity over a wide dynamic range.**
- **The TSKgel Protein A-5PW column showed excellent linearity up to at least 10 g/L for IgG (upper).**
- **No change of column performance was evident after over 2,000 injections (lower).**

Linearity Analysis Comparison

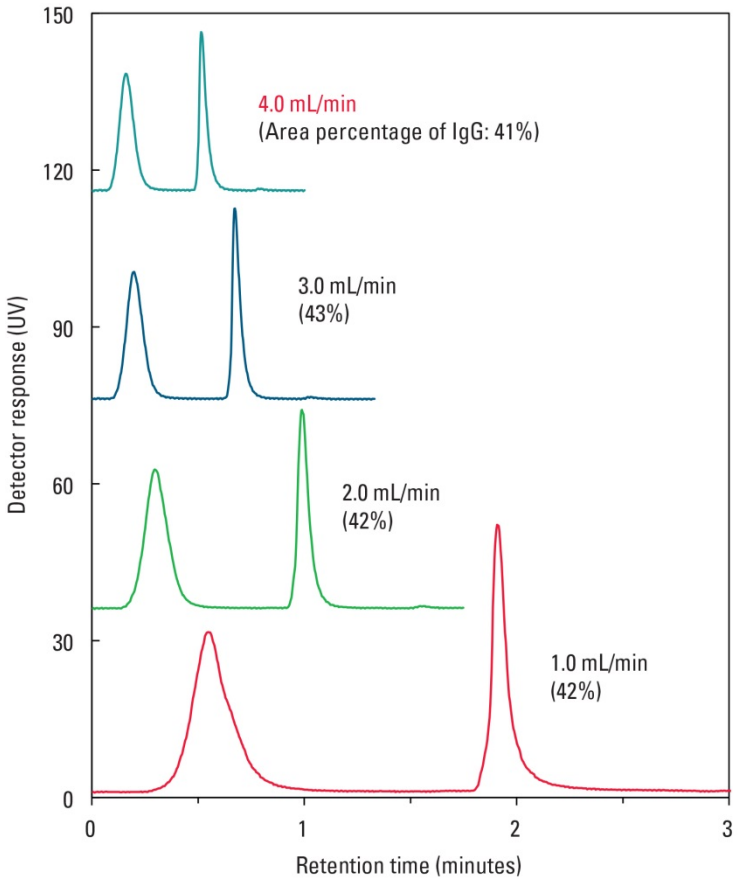


Column:	Competitor Protein A, 4.6 mm ID × 5 cm
Binding buffer:	50 mmol/L sodium phosphate buffer + 150 mmol/L NaCl, pH 7.0
Elution buffer:	50 mmol/L sodium phosphate buffer + 150 mmol/L NaCl, pH 2.5
Stepwise gradient:	0 - 0.5 min: binding buffer 0.5 - 1.5 min: elution buffer 1.5 - 3.0 min: binding buffer
Sample:	polyclonal IgG (0.0125 - 1 g/L; 0.25 - 20 µg)

Conditions for TSKgel Protein A-5PW column are as listed in previous chromatograms.

The competitor Protein A column showed less precision at low concentration of IgG (higher y-intercept of calibration curve).

High Flow Rates for High Throughput Analysis



20 µL of CHO cell supernatant spiked with polyclonal antibody (0.5 mg/mL)

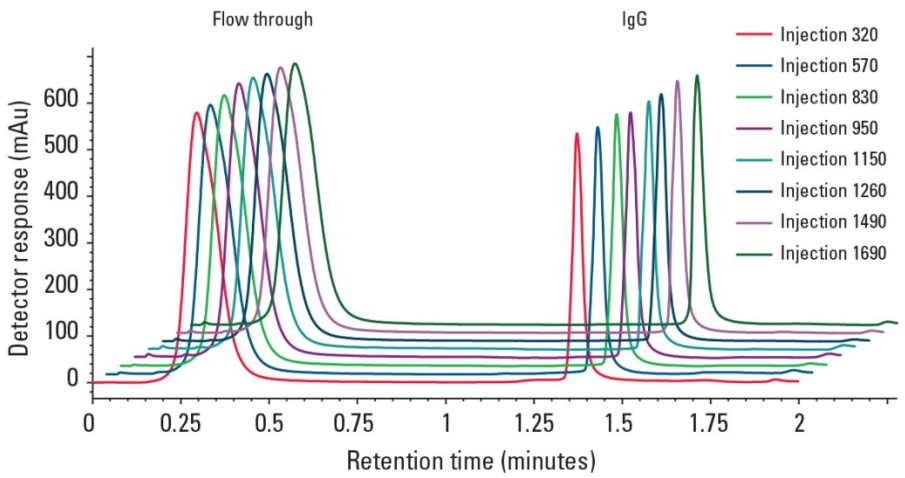
Gradient conditions

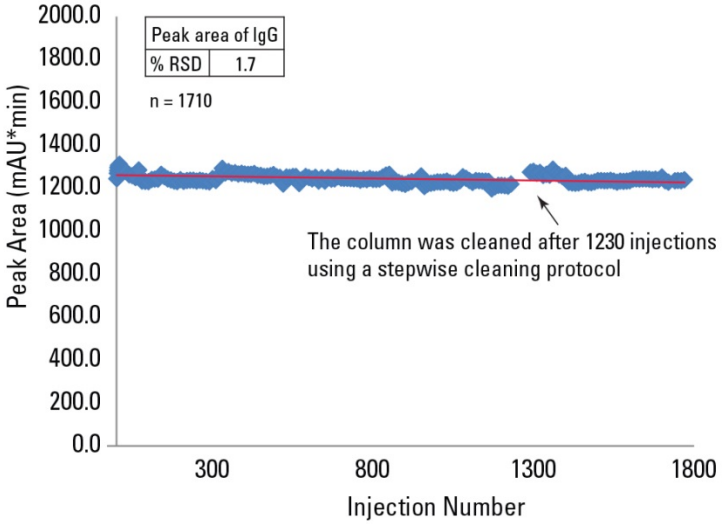
Flow rate (mL/min)	Binding buffer (min)	Elution buffer (min)	Binding buffer (min)
4.0	0-0.25	0.25-0.55	0.55-1.00
3.0	0-0.33	0.33-0.73	0.73-1.33
2.0	0-0.50	0.50-1.10	1.10-2.00
1.0	0-1.00	1.00-2.20	2.20-4.00

20 µL of CHO cell supernatant spiked with polyclonal antibody (0.5 mg/mL)

- **TSKgel Protein A-5PW column shows similar recovery of IgG up to 4.0 mL/min.**
- **Less than 1 minute analysis was possible at 4.0 mL/min with similar peak profile.**

Durability study using CHO crude Feedstock containing IgG_1





- Injection vol.: 5 µL
- Sample: CHO crude feedstock containing IgG_1 (3.2 mg/mL)

- **With hundreds of injections of crude feedstock, the TSKgel Protein A-5PW column did not show any significant change of peak profile.**
- **The column maintains peak area consistency with % RSD of 1.7 after 1710 injections.**

Conclusions

- The TSKgel Protein A-5PW affinity column, containing a protein A ligand with a code-modified hexamer of the C domain, shows unique binding affinities for the IgG samples tested. A further study is required to understand the nature of this interaction.
- The TSKgel Protein A-5PW column showed excellent linearity over a wide dynamic range, which is necessary for the accurate determination of mAb concentration.
- The capability of the TSKgel Protein A-5PW column to run at higher flow rates facilitates high throughput analysis of IgG from a variety of sources without loss in resolution.
- Even with the challenging chromatographic separation of crude feedstock, the TSKgel Protein A-5PW column proved to be robust over hundreds of injections.