avantor™

Technical note

J.T.Baker® Binding affinity of PROchievA™ resin to IgG from different species

J.T.Baker®

It is generally known that protein A, a 42 kDa surface protein of Staphylococcus aureus, has different binding affinities towards antibodies depending on the host species and isotypes due to amino acid sequence variation of the IgG H-chains [1]. BAKERBOND® PROchievA™ affinity resin has an agarose backbone modified with an engineered protein A ligand that is designed to provide high binding capacity and stability for use in mAbs and Fc fusion protein purification [2]. The objective of this study was to evaluate the binding profiles of PROchievA™ resin against IgG from different species to show feasibility of using the resin for different applications.

MATERIALS AND METHODS

A frontal chromatographic technique was used to determine dynamic binding capacity (DBC) at 10% breakthrough according to the methods in Table 1 and 2. Purified IgG from five different species, human, rabbit, goat, bovine, and mouse were loaded onto the PROchievA™ column to determine dynamic binding capacity. Additionally, for human IgG capacity, three samples were tested, which included IgG_4 and two samples of IgG_1. For purification of samples, 5 ml pre-packed PROchievA™ columns were used and 1 ml pre-packed PROchievA™ columns were used for DBC measurement.

To test the binding capacity during a purification process, human IgG_4 sample in clarified cell culture supernatant (3.57 mg/ml) was loaded onto the pre-packed 5 ml column and flowthrough fractions are analyzed to determine DBC at 10% breakthrough. The operating conditions are shown in Table 1 and 2.

Step	Buffer	Volume	Flow rate
Pre-equilibration wash	100mM HAc, pH 3.4	5 CV	1 ml/min
Equilibration	1X PBS, pH 7.4	10 CV	1 ml/min
Sample load	Purified samples (2 mg/ml) in 1X PBS, pH7.4	Until ~15% breakthrough	0.13ml/min (8 min Residence Time)
Wash	1X PBS, pH 7.4	5 CV	1 ml/min
Elution	100mM HAc, pH 3.4	10 CV	1 ml/min
CIP	0.5N NaOH	5 CV	1 ml/min

TABLE 1: Operating condition for DBC measurement with 1 ml pre-packed column. (Samples: human IgG_4, rabbit, goat and bovine IgG)

Step	Buffer	Volume	Flow rate
Pre-equilibration wash	100mM HAc, pH 3.4	5 CV	1 ml/min
Equilibration	50mM Tris, 0.9M NaCl, pH 8.6	10 CV	1ml/min
Sample load	Purified mouse IgG_1 (1 mg/ml) in 50mM Tris, 0.9M NaCl, pH 8.6	Until ~15% breakthrough	0.5 to 0.13 ml/min (2 - 8 min Residence Time)
Wash	50mM Tris, 0.9M NaCl, pH 8.6	5 CV	1 ml/min
Elution	100mM HAc, pH 3.4	10 CV	1 ml/min
CIP	0.5 N NaOH	5 CV	1 ml/min

TABLE 2: Operating conditions for DBC measurement of mouse IgG_1 with 1 ml pre-packed column (Sample: mouse IgG_1)

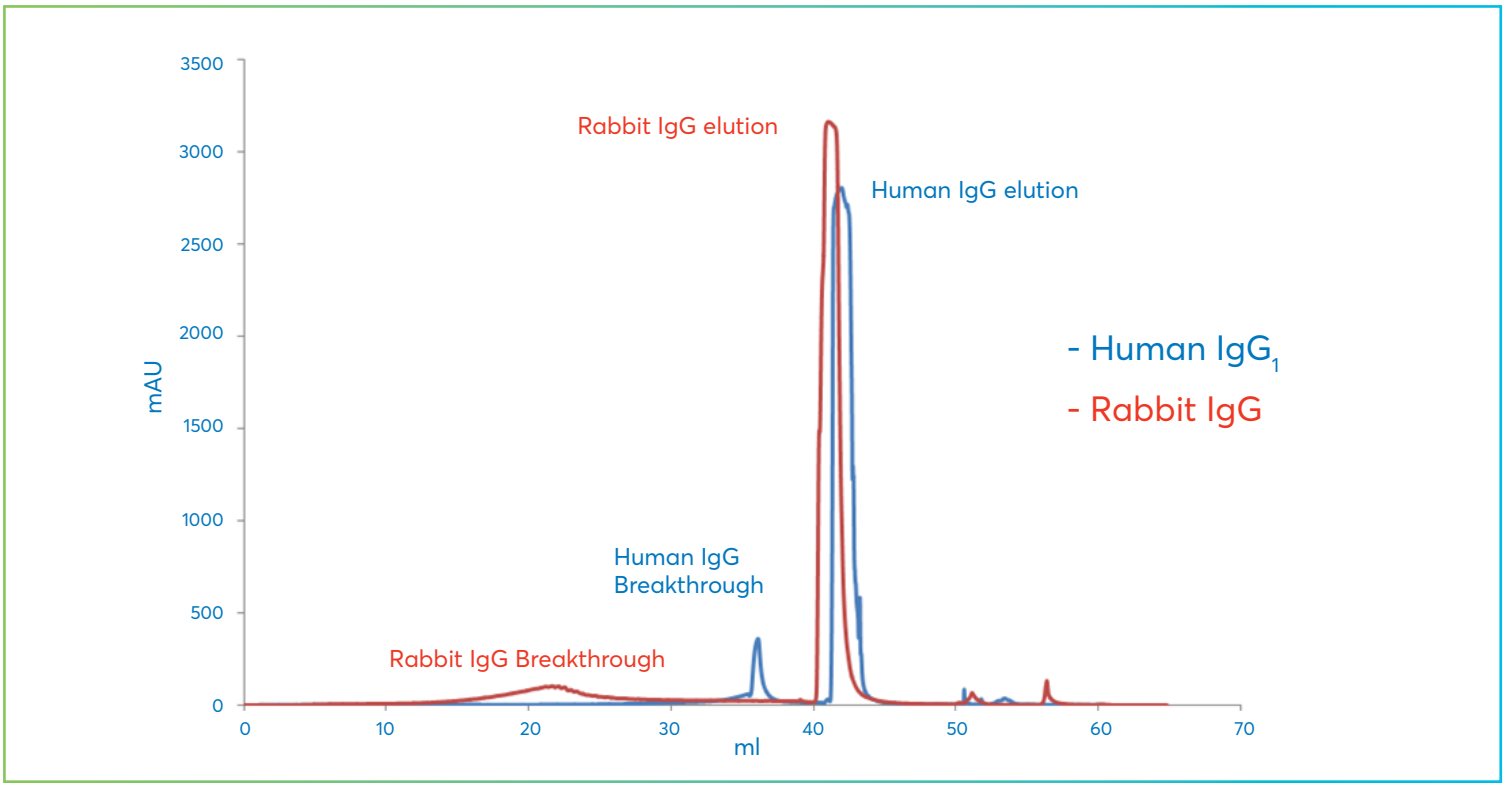

FIGURE 1: Chromatogram of DBC analysis of human IgG and rabbit IgG

AFFINITY TOWARDS VARIOUS IgG

PROchievA™ showed varying degrees of binding affinity for IgG from different species. Chromatogram profile exhibiting such behavior is shown in Figure 1. The DBC values of human IgG_1 and rabbit IgG are significantly different at a same residence time. Table 3 shows the DBC data of PROchievA™ for human IgG, rabbit, goat, bovine and mouse IgG.

IgG	DBC (mg/mL)	Residence time (min)
Human IgG_1 (Sample 1)	68.8	8
Human IgG_1 (Sample 2)	68.9	8
Human IgG_4	55.4	6
Rabbit IgG	36.3	8
Goat IgG	66.0	8
Bovine IgG	61.7	8
Mouse IgG_1	31.6	8

TABLE 3: DBC of PROchievA™ towards various IgG from different species

COMPARISON OF COMMERCIALLY AVAILABLE PROTEIN A RESINS' DBC FOR MOUSE IgG_1

Mouse IgG_1 has various applications, especially for diagnostics and assay development, while finding protein A resin with high capacity for mouse IgG_1 is challenging. Table 4 shows DBC of PROchievA™ at different residence times. To compare the performance, experiments were performed on competitor's new generation protein A resins at 2 min residence time.

Protein A resin	Residence time (min)	Mouse IgG1 DBC (mg/mL)
PROchievA™	8	31.6
	6	30.3
	4	28.3
	2	20.3
Competitor resin 1	2	7.5
Competitor resin 2	2	3.7

TABLE 4: DBC of different agarose protein A resin for mouse IgG_1 sample

CONCLUSION

The Fc-specific BAKERBOND® PROchievA™ showed high dynamic binding capacity for IgG antibodies from different species and two subclasses, IgG_1 and IgG_4. For example, compared to the competitor's resins, the DBC for mouse IgG_1 was 3 to 5 times higher by PROchievA™ at the same process conditions.

Materials used with ordering information

Materials	Avantor part number
PROchievA™ 1 ml column	C789-11
PROchievA™ 5 ml column	C789-18
Sodium Phosphate Dibasic anhydrous	3826
Sodium Phosphate Monobasic monohydrate	3802
Sodium Chloride	3625
Acetic acid	9526
Tris (Base)	4102
TrisHCl	4106
0.5N NaOH	0329

Reference

1. Atkins KL, Burman JD, Chamberlain ES, Cooper JE, Poutrel B, Bagby S, Jenkins AT, Feil EJ, van den Elsen JM: S. aureus IgG-binding proteins SpA and Sbi: host specificity and mechanisms of immune complex formation. Mol Immunol. 2008 Mar;45(6):1600-11. doi: 10.1016/j.molimm.2007.10.021. Epub 2007 Dec 3. PMID: 18061675

2. Avantor: J.T.Baker® BAKERBOND® PROchievA™ Recombinant Protein A, Affinity Chromatography Resin. https://us.vwr.com/store/product/30493609/j-t-baker-sup-sup-bakerbond-sup-sup-prochievatm-recombinant-protein-a-affinity-chromatography-resin

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