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Technical note

J.T.Baker® Specific binding to Fc domain of BAKERBOND® PROchievA™ resin

J.T.Baker®

It is generally known that protein A has different binding affinities to antibodies depending on the host species and isotypes due to amino acid sequence variation of the IgG H-chains [1]. It is also demonstrated that protein A can bind to Fc and VH3 Fab domains. The high affinity of protein A to the Fc domain of IgG has made protein A chromatography the most commonly used capture step in the manufacture of monoclonal antibodies (mAbs). However, interaction with VH3 domain is not preferred for mAb manufacturing [2]. BAKERBOND® PROchievA™ affinity resin has an agarose backbone with engineered protein A ligand, where the ligand is designed to provide a high binding capacity, stability and specificity to the Fc domain of IgG for use in mAbs and Fc fusion protein purification [3]. In this study, we compared the binding affinity of PROchievA™ to mAb, Fab, F(ab')2 and Fc fragments. Lack of Fab and F(ab')2 binding to PROchievA™ demonstrates Fc domain specificity of the engineered ligand. Because of the specific affinity of PROchievA™ towards the Fc region, it can be used in various applications such as collecting target Fab fragments from flow-through fraction or collecting purified mAbs from elution pool without fragment impurities

MATERIALS AND METHODS

In order to show the specific affinity of PROchievA™ to Fc region, IgG was digested with papain and pepsin enzymes to create Fab, F(ab')2, and Fc fragments. Graphic explanation of papain and pepsin enzyme working mechanism is shown in Figure 1.

25mg of papain or pepsin digested IgG was loaded onto 1ml pre-packed PROchievA™ column and purified using the conditions shown in Table 1. Fractions of load, flow-though, wash, and elution pools were collected and analyzed with SDS-PAGE to identify the binding and elution of Fab and Fc fragments. All experiments were performed at 2-8°C including the cleaning of the column with 0.5N NaOH between each run.

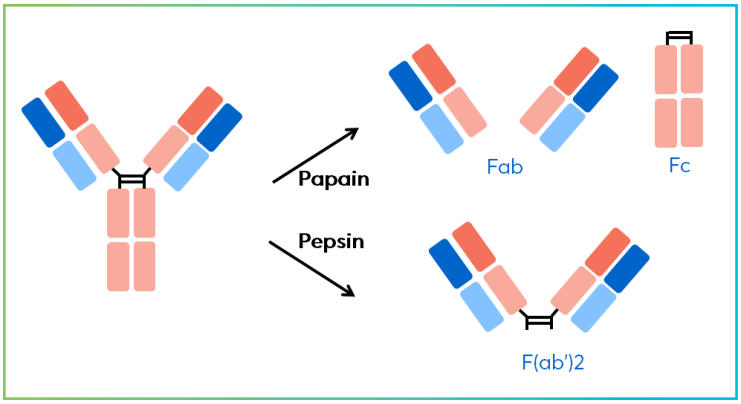
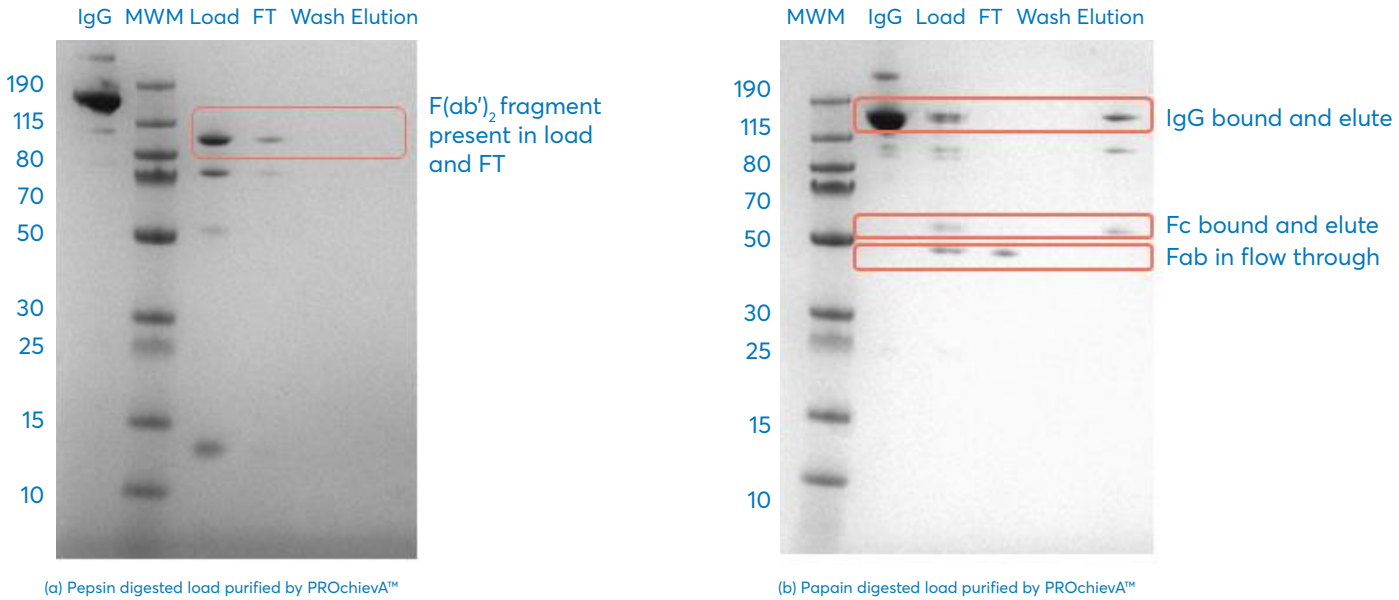


FIGURE 1: Enzyme mechanism of papain and pepsin

Step	Buffer	Volume	Flow rate
Strip	100mM acetic acid pH 3.4	5CV	0.25ml/min
Equilibrium	1X PBS pH 7.4	10CV	0.25ml/min
Sample load	Diluted in 1X PBS pH7.4	N/A	0.25ml/min (4 min Residence Time)
Wash	1X PBS pH 7.4	5CV	0.25ml/min
Elution	100mM acetic acid pH 3.4	10CV	0.25ml/min
CIP	0.5N NaOH	5CV	0.25ml/min

TABLE 1: Operating condition for purification of Fab and Fc fragments using 1ml column



(a) Pepsin digested load purified by PROchievA™

(b) Papain digested load purified by PROchievA™

FIGURE 2: SDS-PAGE analysis of pepsin and papain digested IgG purified using PROchievA™

SPECIFIC AFFINITY TO FC DOMAIN

The SDS-PAGE analysis of the chromatographic purifications of IgG digested by either pepsin or papain is shown in Figure 2. Results of pepsin digested IgG are shown in Figure 2(a) where F(ab')2 fragments are collected in flow-through due to no affinity towards resin. Because the Fc regions are completely digested by pepsin, no protein is observed in the elution fraction. The results of papain digested IgG are shown in Figure 2(b) where Fab fragments are collected in flow-through due to no affinity towards resin. Elution fraction shows Fc fragment and undigested IgG, which demonstrates the specific affinity of PROchievA™ to Fc region.

CONCLUSION

PROchievA™ resin shows specific affinity towards the Fc region of IgG resulting in an efficient purification of mAb and Fc-fusion protein. This property can also be used for removal of fragment impurities while purifying IgG or separation of Fab fragments from IgG by collecting flow-through.

Materials used with ordering information

Materials	Avantor part number
PROchievA™ 1ml column	C789-11
PROchievA™ 5ml 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, Karen L., et al. "S. Aureus IgG-binding proteins SpA and Sbi: Host specificity and mechanisms of immune complex formation." Molecular Immunology. 2008;45(6): 1600–1611., doi:10.1016/j.molimm.2007.10.021.

2. Bach, Julia, et al. "Differential binding of heavy chain variable domain 3 antigen binding fragments to protein A chromatography resins" Journal of Chromatography A, 1409(2015) 60-69, doi: 10.1016/j.chroma.2015.06.064

3. Avantor "J.T.Baker BAKERBOND® PROchievA™ recombinant protein A affinity chromatography resin." Accessed 09 Jan 2021.

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