

# Platform for purification of V<sub>H</sub>H-type antibody fragments

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## Abstract

Protein A affinity chromatography resins have for a long time been used to purify monoclonal antibodies (mAbs) by the bioprocess industry at all scales. The binding of protein A mainly takes place between CH2 and CH3 in the Fc region of the mAb (constant domains 2 and 3 of the Ab heavy chains). However, it is known that protein A also can bind to V<sub>H</sub>3 sequences located on the variable heavy chain of the Fab region. Here we will show examples of purification of mAb derived fragments, including bispecific antibodies (BsAb) and V<sub>H</sub>H, with a focus on binding characteristics, e.g., binding capacities, elution conditions, and purification performance. It will be demonstrated that a good yield of V<sub>H</sub>H can be purified directly from a high load of an *E. coli* harvest.

## Introduction

Traditionally protein A chromatography has been used for purifying mAb using the interaction with the Fc region of IgG. Today, we see a greater diversity of mAb derived molecules (Fig 1), include some that lack the Fc region which require resins with ligands binding to other parts of the molecule (Fig 2).



Fig 1. Example of the diversity of mAb derived molecules.

Fig 2. Different interaction points on mAb for alternate purification possibilities.

One possibility is to use affinity resins for polishing product specific impurities, e.g., bispecific antibodies with a κ chain on one arm and λ chain on the other arm, a resin with binding to either κ (Capto™ L) or λ (LambdaFabSelect) chains could be utilized. Another interaction that is possible to use for polishing on product related impurities, as well as for capture, is the V<sub>H</sub>3 interaction displayed by protein A resins. However, protein A is not as base stable as more modern resins, MabSelect SuRe™ and MabSelect Prisma™ in particular, but the modern resins have not been fully evaluated for the V<sub>H</sub>3 interaction. Table 1 shows the V<sub>H</sub>3-determining amino acids and their positions (Kabat nomenclature).

Table 1. V<sub>H</sub>3-determining amino acids.

VH	H15	H17	H19	H57	H59	H64	H65	H66	H68	H70	H81	H82a	H82b
VH3	G	S	R, K	K, I, T	Y	K	G	R	T	S	Q	N, G	S
Kabat	HFR1			CDR-H2				HFR3					

## Results

In Figure 3, three different columns, MabSelect™, MabSelect SuRe™, and MabSelect Prisma™, were loaded with purified V<sub>H</sub>H fragments and eluted with acidic pH. MabSelect™ and MabSelect Prisma™ show the same chromatographic behavior while MabSelect SuRe™ displays a weaker interaction, if any, with the fragment.

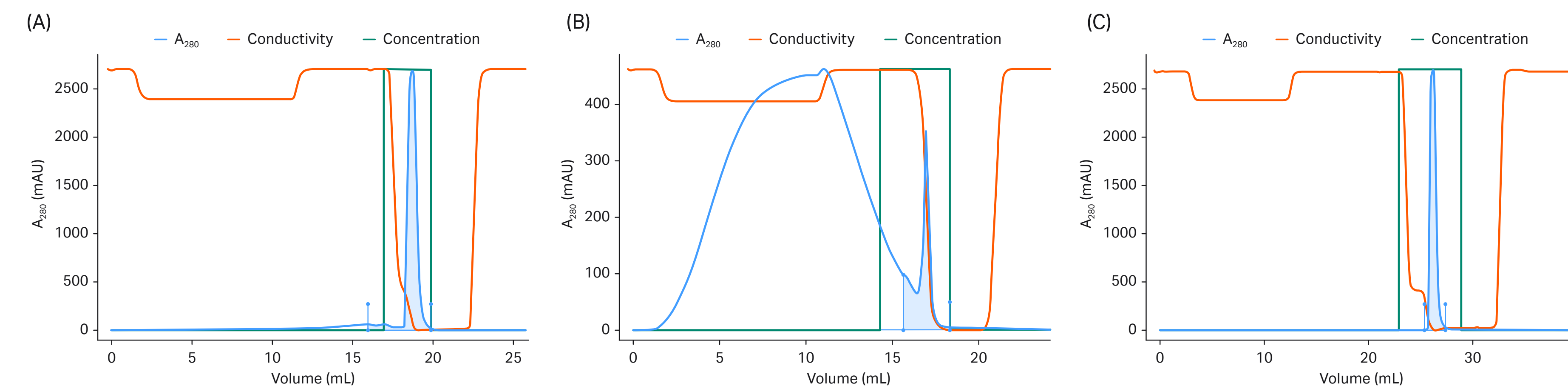


Fig 3. Binding of a V<sub>H</sub>H fragment to (A) MabSelect™, (B) MabSelect SuRe™, and (C) MabSelect Prisma™.

V <sub>H</sub> H sequence	MabSelect™	MabSelect SuRe™	MabSelect Prisma™
T57	Yes	Yes	Yes
T57K	Yes	No	Yes
T57I	No	No	No
T57E	No	No	No
N82aT, S82bD	No	No	No
T57P	Yes	No	Yes
T57S	Yes	No	Yes
T57R	Yes	No	Yes
K64R	Yes	Yes	Yes
K64T	Yes	Yes	Yes
K64E	Yes	N/A	Yes
G65D	No	No	No
S70T	Yes	No	Yes
S82bD	No	No	No
S82bG	Yes	Yes	Yes

Table 2. Interaction of MabSelect™, MabSelect SuRe™, and MabSelect Prisma™ to V<sub>H</sub>H fragments, i.e., mimic of the V<sub>H</sub> region of IgG, mutated at different positions. The data in the table demonstrates that MabSelect SuRe™ can interact with the V<sub>H</sub>3 region in some cases, but MabSelect™ and MabSelect Prisma™ are less sensitive to sequence variation.

## Materials and methods

The V<sub>H</sub>H's were fermented in *E. coli* in shake flasks or in 5 L fermentors. In Table 2, 1 mL HiTrap™ packed with the different resins. In Figure 4, 150 mL harvest of the T57 V<sub>H</sub>H material from a 5 L fermentor was loaded to a Tricorn™ 5/50 column packed with 1 mL MabSelect Prisma™ at 6 minutes residence time. In Table 3 and 4 the same column was used to determine the breakthrough capacity and elution pH.

## Purification of a V<sub>H</sub>H fragment on MabSelect Prisma™

The binding capacity for V<sub>H</sub>H and Fab was determined by frontal analysis and elution pH using a pH gradient from pH 6 to 3 in a Tricorn™ 5/50 column packed with 1 mL MabSelect Prisma™, shown in Table 3. 150 mL *E. coli* harvest containing 0.2 g/L V<sub>H</sub>H (30 g V<sub>H</sub>H/L resin) was loaded at 6 minutes residence time to a MabSelect Prisma™ column (CV 1 mL) as displayed in Figure 4. The fragment was eluted in 50 mM Na-acetate pH 3.5.

In Figure 4 the MabSelect Prisma™ capture of the V<sub>H</sub>H from an *E. coli* harvest is shown.

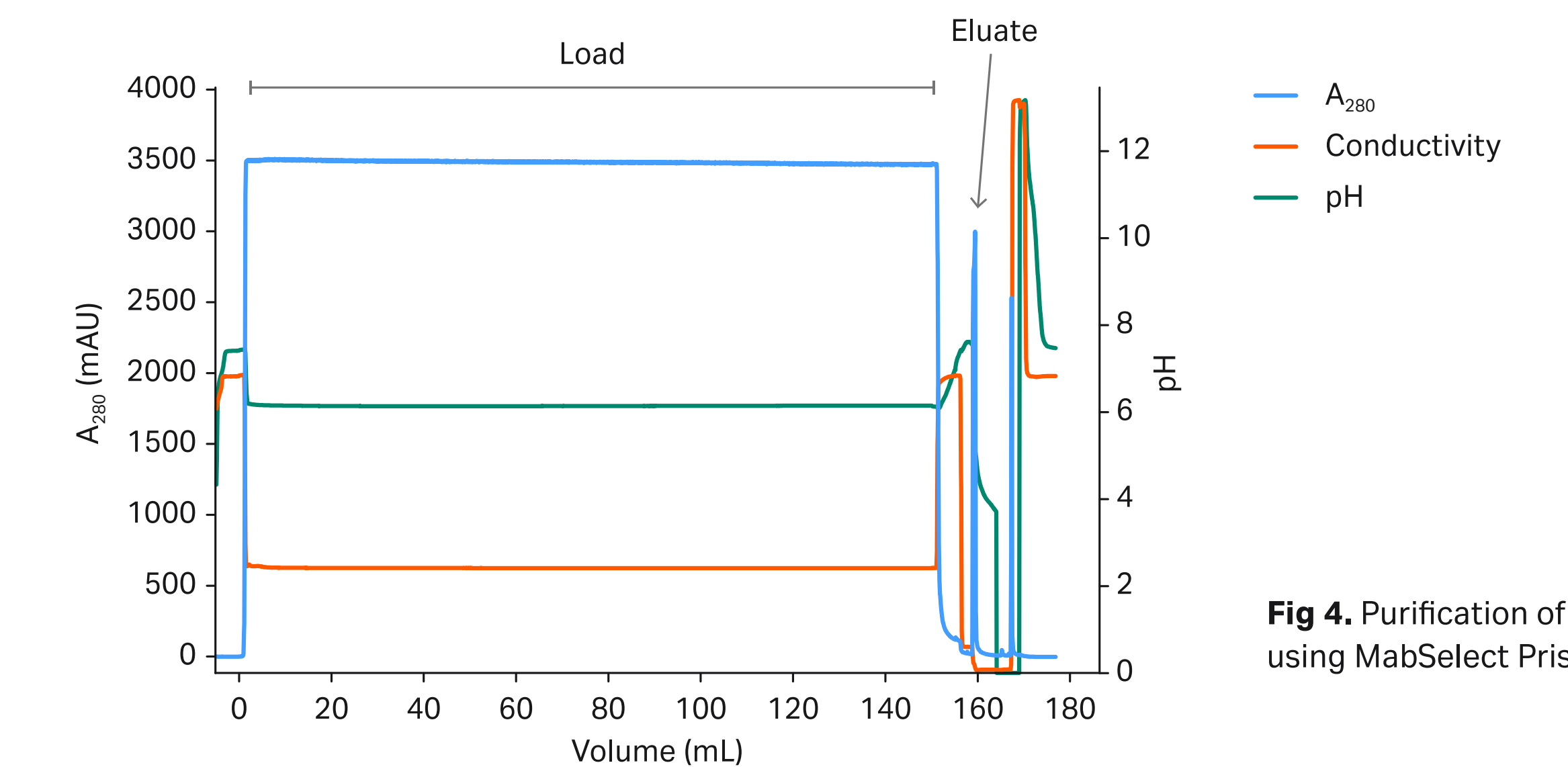


Fig 4. Purification of V<sub>H</sub>H using MabSelect Prisma™.

Table 3. MabSelect Prisma™ capacity and the V<sub>H</sub>H elution pH

Entity	Q <sub>b10</sub> (g/L)	Q <sub>b10</sub> (mM)	Elution pH
V <sub>H</sub> H	42	2.7	3.8

Table 4. Analytical data from the purification in Figure 4.

Sample	Pool volume (CV)	eHCP (ppm*)	Leached ligand (ppm**)
Load	NA	4252500	NA
Elution pool	1	200	10

\*ng eHCP/mg V<sub>H</sub>H, \*\*ng leached ligand/mg V<sub>H</sub>H

## Conclusion

The modern base stabilized protein A resin MabSelect Prisma™ can be used for purification of molecules lacking the Fc region by utilizing the V<sub>H</sub>3 interaction, resulting in:

- High capacities
- High purities are achieved for *E. coli* harvest containing V<sub>H</sub>H (4.3 logs reduction of HCP)