# Platform for purification of $V_HH$-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 $V_H3$ 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 $V_HH$, with a focus on binding characteristics, e.g., binding capacities, elution conditions, and purification performance. It will be demonstrated that a good yield of $V_HH$ 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).

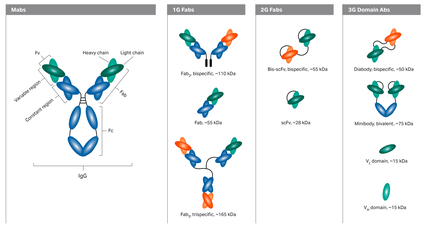


**Fig 1.** Example of the diversity of mAb derived molecules.

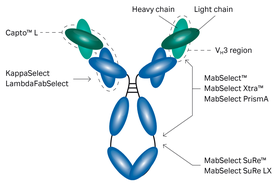


**Fig 2.** Different interaction points on mAb for alternate purification possibilities.

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 $V_H3$ 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 $V_H3$ interaction. Table 1 shows the $V_H3$-determining amino acids and their positions (Kabat nomenclature).

**Table 1.** $V_H3$-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 $V_HH$ 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.

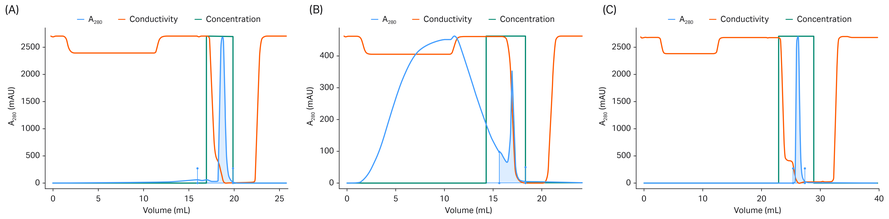


**Fig 3.** Binding of a $V_HH$ fragment to (A) MabSelect, (B) MabSelect SuRe, and (C) MabSelect PrismA.

| $V_HH$ 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 $V_HH$ fragments, i.e., mimic of the $V_H$ region of IgG, mutated at different positions. The data in the table demonstrates that MabSelect SuRe can interact with the $V_H3$ region in some cases, but MabSelect and MabSelect PrismA are less sensitive to sequence variation.

### Purification of a $V_HH$ fragment on MabSelect PrismA

The binding capacity for $V_HH$ 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 $V_HH$ (30 g $V_HH$/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 $V_HH$ from an *E. coli* harvest is shown.

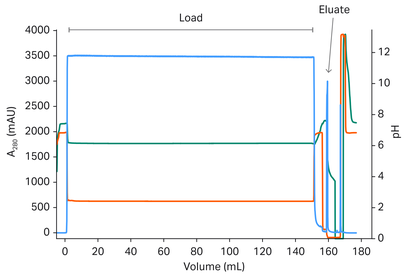


**Fig 4.** Purification of $V_HH$ using MabSelect PrismA.

**Table 3.** MabSelect PrismA capacity and the $V_HH$ elution pH

| Entity | $Q_{b10}$ (g/L) | $Q_{b10}$ (mM) | Elution pH |
|---|---|---|---|
| $V_HH$ | 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 $V_HH$. **ng leached ligand/mg $V_HH$

## Materials and methods

The $V_HH$'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 $V_HH$ 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.

## Conclusion

The modern base stabilized protein A resin MabSelect PrismA can be used for purification of molecules lacking the Fc region by utilizing the $V_H3$ interaction, resulting in:

- High capacities
- High purities are achieved for *E. coli* harvest containing $V_HH$ (4.3 logs reduction of HCP)

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CY11918-10Sep20-PT