APPLICATION NOTE

Differential Fab Binding to GORE® Protein Capture Devices

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Background

The binding affinity of Fab fragments to protein capture devices is influenced by the amino acid sequence in the VH3 region, particularly at residue 57. Previous studies, including those by Bach et al., have shown differential binding to MabSelect SuRe™ (MSS) based on the amino acid at this position. This study aims to evaluate the binding potential of GORE® Protein Capture Devices (GPCD) compared to MSS using Herceptin Fab heavy chain sequences with point mutations at residue 57.

Purpose

To assess the Fab binding potential of GPCD relative to MabSelect SuRe using Herceptin Fab heavy chain sequences with specific point mutations at residue 57.The subsequent downstream purification processing of multiple samples can take multiple days to a week using standard resins. The long residence times and slow flow rates associated with standard resins on standard FPLC systems translate to long purification cycle times and low productivity.

Experimental Plan

Method

1. Convert Herceptin hG1 to Fab heavy chain format.
2. Introduce three amino acid point mutations at residue 57: Threonine (T57), Proline (T57P), and Isoleucine (T57I) as shown in Figure 1.

Figure 1. Herceptin CDR H2 Sequence and amino acid 57 of interest

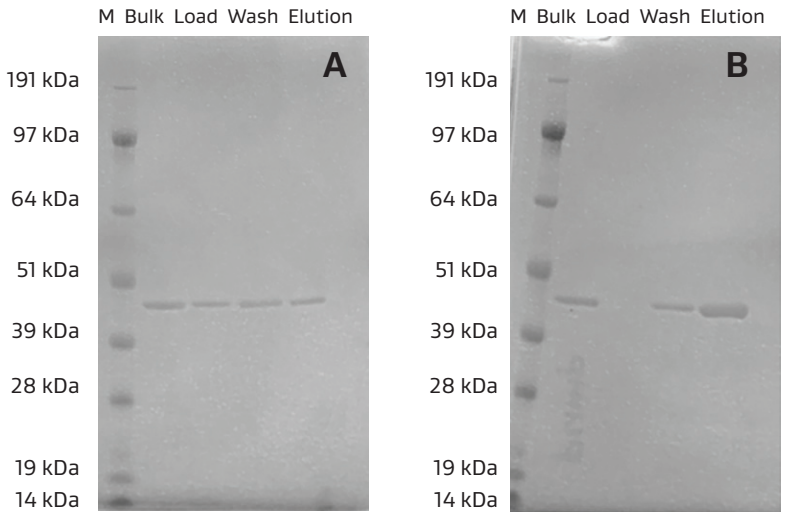
3. Perform batch binding experiments with agarose materials and LC experiments comparing GPCD to various agarose resins.
4. Dilute bulk purified T57 or T57X material into PBS at 2.6 mg/purification per device. Perform bind and elute cycle on GPCD and MSS columns.
5. Analyze binding on SDS-PAGE gel comparing bulk, load, wash, and elution

Results

Experiment 1: Threonine (T57)

As shown in Figure 2 in the elution, both the GPCD and MSS bind to some degree with threonine at residue 57.

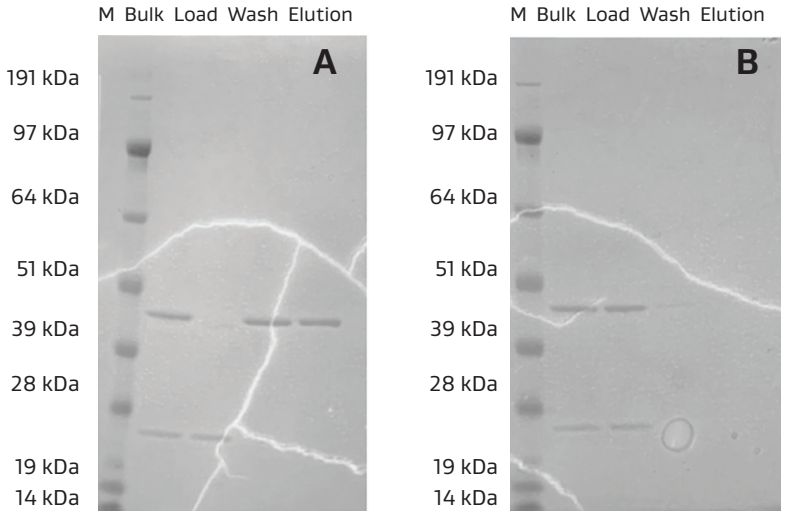
Figure 2. A) GORE Protein Capture Device binding of Fab fragment with T57, B) MSS binding of Fab fragment with T57.



Experiment 2: Proline (T57P)

As shown in Figure 3 in the elution, GPCD exhibited binding of purified Fab material, while MSS did not bind to Fab with Proline at residue 57.

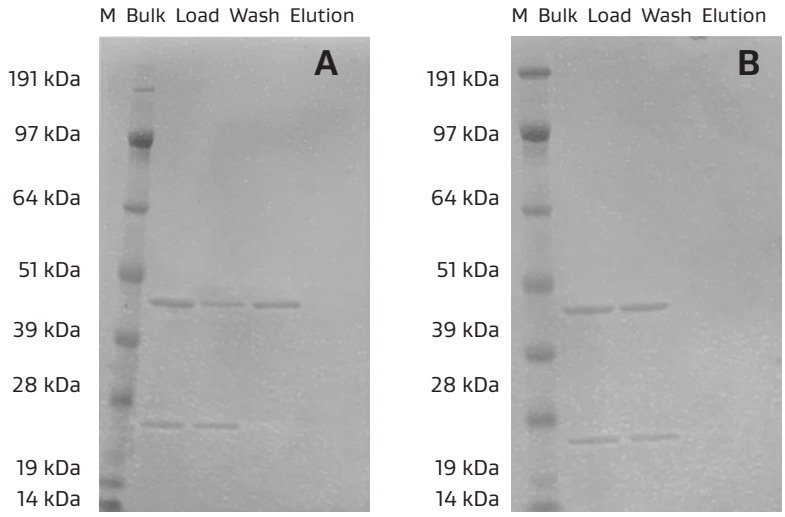
Figure 3. A) GORE Protein Capture Device binding of Fab fragment with T57P, B) MSS binding of Fab fragment with T57P.



Experiment 3: Isoleucine (T57I)

As shown in Figure 4 Neither GPCD nor MSS exhibited binding of purified Fab material with isoleucine at residue 57.

Figure 4. A) GORE Protein Capture Device binding of Fab fragment with T57I, B) MSS binding of Fab fragment with T57I.



A summary of the binding relative to the amino acid at position -57 is shown in Table 1.

Table 1. Amino Acid Point Mutation and Binding Profile

AA (-57)	GPCD	MSS
Threonine (T57)	Y	Y
Proline (T57P)	Y	N
Isoleucine (T57I)	N	N

Discussion

The findings can guide the design of Fab fragments to either enhance or eliminate binding to specific protein capture devices based on the amino acid at residue 57. This can be particularly useful in antibody-based therapeutic development, where specific binding profiles are required for efficacy and safety. Specifically, the results can be used in designing and optimizing protein purification of Fab fragments where binding to the high-productivity GORE Protein Capture Device with Protein A is desired. By understanding the binding behavior of different amino acids at residue 57, purification protocols can be tailored to optimize yield and purity of Fab fragments.

The results provide a reference for researchers planning experiments involving protein capture devices, helping them choose appropriate amino acid mutations to achieve desired binding profiles. These practical applications can help streamline protein purification processes, enhance molecule design, and improve experimental outcomes in various biotechnological and pharmaceutical contexts.

Conclusions

The GORE Protein Capture Device exhibits binding dependent on the amino acid sequence at residue 57 in the VH3 region. Fab binding profiles can be manipulated by altering the amino acid at residue 57. Experimental results confirm that literature references can guide molecule design to eliminate Fab binding irrespective of the ligand used for Protein A purification.

References

J. Bach *et al.*, J. Chromatogr. A 1409 (2015) 60-69.

Gore PharmBIO Products

Our technologies, capabilities, and competencies in fluoropolymer science are focused on satisfying the evolving product, regulatory, and quality needs of pharmaceutical and bioprocessing customers, and medical device manufacturers. GORE® Protein Capture Devices with Protein A, like all products in the Gore PharmBIO Products portfolio, are tested and manufactured under stringent quality systems. These high-performance products provide creative solutions to our customers' design, manufacturing, and performance-in-use needs

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