

Increasing Purity on ProSep®-vA Affinity Chromatography Media using an Intermediate Wash Step

Introduction

ProSep-vA protein A affinity chromatography media enables high-throughput purification for monoclonal antibodies (MAbs) together with long lifetime and broad process flexibility. ProSep-vA media, as with all chromatography media, can exhibit a degree of non-specific binding¹, which is typically characterized by co-elution of host cell proteins (HCP) together with the MAb. Typically the levels of non-specific binding (NSB) to ProSep-vA media are very low and the purity of the eluate can routinely exceed 96%, and the residual HCP levels can readily be cleared by subsequent purification steps. In situations where lower HCP levels are desirable, an intermediate wash step can be used. This is aimed at selecting conditions for the post load wash, which will elute HCP and other contaminants, while leaving the MAb bound. Subsequent elution of the MAb will result in a higher purity product pool.

While ProSep-vA media provides the benefits of rigidity and an open pore structure enabling high flow and longer bed operation, the controlled pore glass (CPG) base matrix sometimes exhibits a relatively higher NSB level compared to the more compressible agarose based matrices. Selecting the right intermediate wash buffer, however, can result in HCP levels at least equal to, if not lower than, levels obtained with agarose-based media.

Choice of Intermediate Washes to Reduce Non-Specific Binding

Non-specific binding is usually due to either ionic or hydrophobic interaction with the base matrix or immobilization chemistry. The aim is to modify the post-load wash buffer in such a way as to disrupt these interactions, which will elute the non-specifically bound contaminants without prematurely eluting the MAb.

Several approaches have proven effective. These include selecting a pH for the intermediate wash buffer that is between the loading and the elution buffers, and/or the

inclusion of salt, detergents or amino acids such as arginine².

Recently, the use of buffer combinations consisting of salts and detergents, salts and solvents, salts and polymers, and high Tris buffer concentrations have proven to be effective³. These latter methods are the subject of US Patent 6,870,034 (and its counterparts) to which Millipore has been granted a non-exclusive license by Genentech, Inc. Millipore has the right to grant a sublicense to its customers of ProSep-vA chromatography media who wish to practice the methods claimed in the licensed patent.

Systematic Approach to Intermediate Wash Buffer Selection

While evaluating a broad range of wash options may help in determining the optimum solution for a particular application, a systematic approach to buffer selection with the simplest options evaluated first, as outlined below, is recommended.

Step 1 — Intermediate pH Wash

For the first step, it is best to evaluate a pH for the post-load wash that is intermediate between that of the loading and elution buffers. For maximum removal of non-specifically bound material, the pH of the intermediate wash should be as low as possible, but not so low as to initiate premature elution of the MAb. For example, if the loading pH is pH 7.5 and the elution pH is 4.0, then an intermediate wash buffer pH of 5.0 might be optimal. At the same time as investigating the pH of the wash buffer, it may also be beneficial to investigate the choice of buffer salt. For example, in the case illustrated in Figure 1, citrate was more effective than acetate in reducing HCP levels. The ability to employ an intermediate pH wash is very much dependent on the properties of the particular MAb. While the effectiveness of this approach is clearly illustrated in Figure 1, in other cases it may not be applicable because of loss in yield due to premature MAb elution.

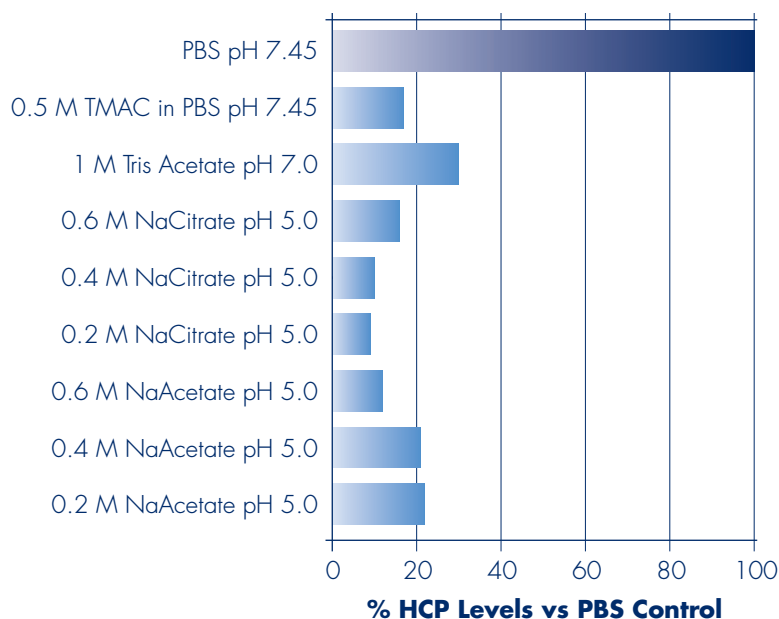
Step 2 — Single-component Buffer Additions

In the case where an intermediate pH wash is not practical or is insufficient alone to attain required HCP levels, then the second step is to evaluate the addition of a salt, amino acid, detergent or solvent to the intermediate wash buffer. Table 1 lists suggested additives and concentrations for initial screening

Note that once a buffer additive is proven effective, the concentration can be further optimized.

Figure 1.

Use of Intermediate pH Buffer to Reduce HCP Levels



Percentage HCP levels in the ProSep-vA High Capacity elution following the use of different intermediate washes as indicated, relative to a PBS pH 7.45 control. Feedstock was a MAb expressing CHO cell culture. High concentration Tris HCl or Tris Acetate (0.5 – 1.0M) are alternative buffers that may also be evaluated.

Table 1.

Single-component Buffer Additives

Single-component Buffer Additive	Concentration
Salts — NaCl, Na ₂ SO ₄	0.5–1.0 M
Detergents — Tween® 20	0.5%
Solvents — Hexylene glycol	10–20%
Polymers — Polyethylene/Polypropylene glycol	10–20%
Amino Acids — Arginine, Glycine	0.5 M

Step 3 — Multi-component Buffer Addition

If steps 1 or 2 are insufficient to reach the required HCP levels, then the next step is to explore the use of multi-component buffer additions where salt and detergents, salt and solvents, or salt and polymers are used in combination. Suggestions for such combinations are listed in Table 2.

Figures 2 and 3 show data illustrating the effectiveness of these combinations.

Table 2.

Multi-component Buffer Additives

Multi-component Buffer Additive

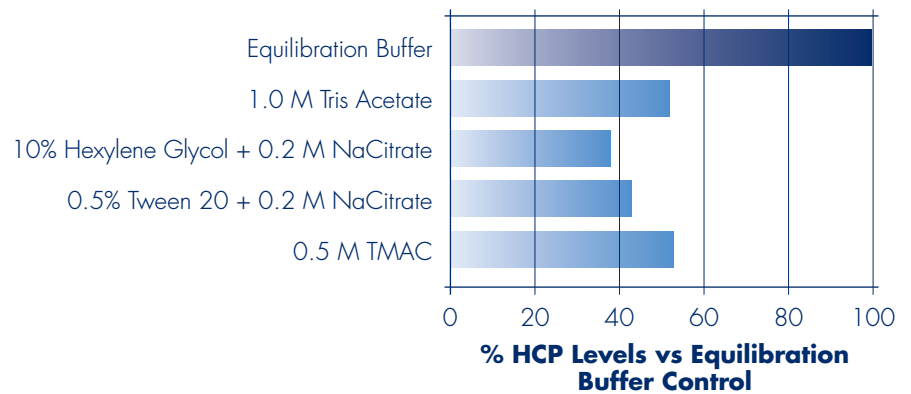
0.5–1.0 M NaCl plus 0.1–1% Tween

0.5–1.0 M NaCl plus 10–20% Hexylene Glycol

0.5–1.0 M NaCl plus 10–20% Polyethylene/ Polypropylene Glycol

Figure 2.

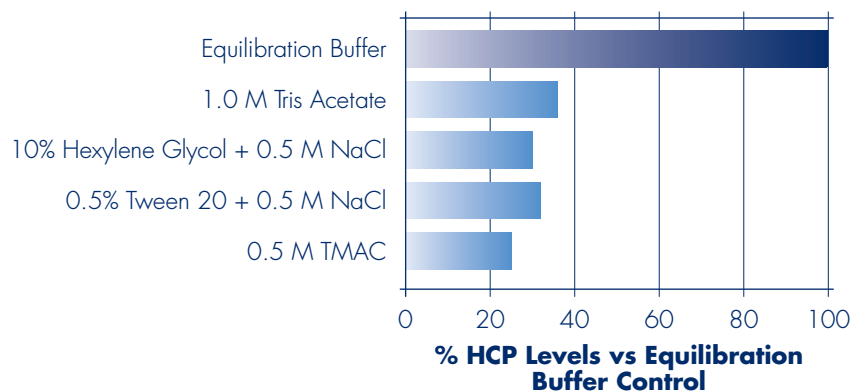
Effect of Multi-component buffers in reducing HCP levels (a)



Comparison of different buffer additives in combination with 0.2M Citrate buffer in reducing HCP levels. Data taken from US Patent 6,870,034. E26 feedstock.

Figure 3.

Effect of Multi-component buffers in reducing HCP levels (b)

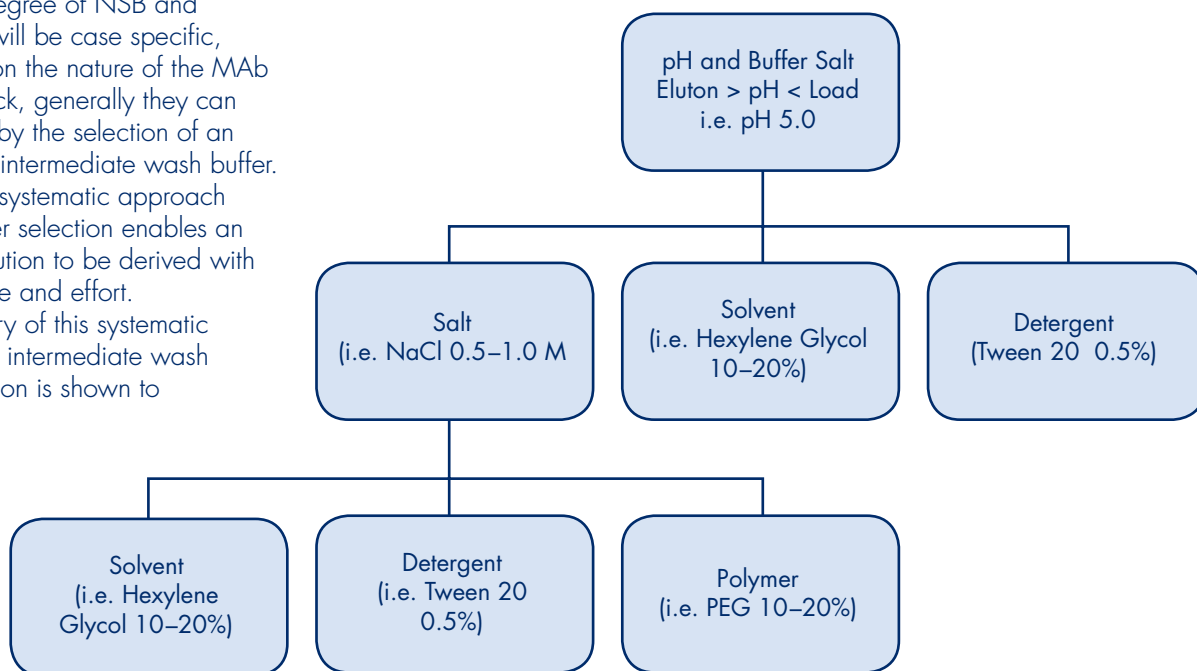


Comparison of different buffer additives in combination with 0.5M NaCl. Data taken from US Patent 6,870,034. Trastuzumab feedstock.

Summary

While the degree of NSB and HCP levels will be case specific, depending on the nature of the MAb and feedstock, generally they can be reduced by the selection of an appropriate intermediate wash buffer. Following a systematic approach to such buffer selection enables an optimum solution to be derived with minimum time and effort.

A summary of this systematic approach to intermediate wash buffer selection is shown to the right.



Note

The references to ProSep-vA chromatography media in this technical brief apply to both ProSep-vA Ultra and ProSep-vA High Capacity media.

Applications Support

Should you have any questions relating to either the use of intermediate washes, including a sublicense to US Patent 6,870,034 or any other aspects of using ProSep-vA chromatography media, Millipore's Chromatography Application Scientists are ready to assist you. Please contact your local Millipore representative for more details on how to access this support.

References

1. Goding, J. in *Monoclonal Antibodies: Principles and Practice*, 3rd Edition, Academic Press, 1996.
2. Millipore Technical Brief TB1024EN00, (2005) Improving Purity on Protein A Affinity Chromatography Media through use of an Arginine Intermediate Wash Step.
3. Breece, T.N., Fahrner, R.L., Gorrell, J.R., Lazzareschi, K.P., Lester, P.M., Peng, D. (2005) Protein Purification. US Patent 6,870,034.

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