

PAB — An Enhanced Sanitization Option for ProSep® Protein A Affinity Chromatography Media

INTRODUCTION

Protein A chromatography media, including ProSep-vA and ProSep Ultra Plus media, require sanitization between batches as well as prior to storage. While stable at low pH, protein A has limited stability at high pH. Even the new protein A variants with improved caustic stability may exhibit a noticeable loss in capacity at the higher NaOH concentrations and/or longer contact times that may be required for effective sanitization.

A solution of 2% benzyl alcohol in 0.1 M sodium acetate at pH 5.2 (+/- 0.5) is frequently used for sanitization of ProSep protein A affinity media. This solution has the advantage of also being suitable for long term storage. While effective as a sanitant, a 24 hour exposure time may be necessary to meet the required microbial log reduction values (LRV). If the column is in storage during this period, extended sanitization time is not an issue. However with a rising market need for monoclonal antibody therapeutics, demands on manufacturing throughput and productivity are increasing. In many instances, columns are not put in storage but immediately reused. In such cases, a shorter sanitization cycle would be extremely beneficial.

A new sanitant solution, PAB (120 mM phosphoric acid, 167 mM acetic acid, 2.2% benzyl alcohol), has been developed to address the need for shorter sanitization times. PAB offers the best combination of microbial kill effectiveness, resin stability and process acceptability.

It exploits the low pH stability of both protein A and the ProSep base matrix by combining phosphoric and acetic acids together with benzyl alcohol. In addition to significantly reducing the sanitization step time, PAB also has the advantage of not introducing novel chemical species into the process. With PAB, sanitization times may be reduced to 2–3 hours.

In comparison studies, PAB is shown to be at least as effective a sanitant as 0.5 M NaOH and significantly more effective than 0.1 M NaOH.

The introduction of PAB as an alternative sanitant solution adds greater flexibility to process design using ProSep chromatography media.



PAB — SANITIZATION EFFECTIVENESS

During development, testing in batch mode started in free solution and then progressed to testing in the presence of naïve and then finally process-challenged resin. No difference in PAB sanitant effectiveness was noted between the different approaches.

The improved microbial kill kinetics of PAB compared to pH 5.0 buffered 2% benzyl alcohol is shown in Table 1. The challenge was performed in the presence of naïve resin at a “worst case” processing temperature of 15 °C, using three different challenge organisms including a mold (*Aspergillus niger* spore preparation), a yeast (vegetative *Candida albicans*) and a bacterial mix (*Staphylococcus aureus*, *Burkholderia cepacia*, *Pseudomonas aeruginosa*).

Table 1. Microbial Kill (Log Reduction Values) in Presence of Naïve Resin at 15 °C

Hours at 15 °C	Trial	LRVs compared to PBS control			
		2 hr	6 hr	10 hr	24 hr
<i>Aspergillus niger</i> spores					
2% benzyl alcohol pH 5.0	1	-0.07	0.02	0.11	0.11
	2	<0.3	0.5	0.14	0.45
PAB	1	4.11	3.13	>4.71	>5.04
	2	3.12	>5.26	>5.02	>5.28
<i>Candida albicans</i>					
2% benzyl alcohol pH 5.0	1	1.66	3.79	>4.41	>4.13
	2	0.22	0.45	1	2.34
PAB	1	>4.41	>4.48	>4.41	>4.13
	2	>5.43	>5.53	>5.38	>5.66
Bacteria mix*					
2% benzyl alcohol pH 5.0	1	5.81	>6.63	>6.63	>6.63
	2	2.28	4.64	5.65	5.8
PAB	1	>6.51	>6.63	>6.63	>6.63
	2	>6.48	>6.62	>6.82	>6.5

PAB solution was 120 mM phosphoric acid, 167 mM acetic acid, and 2.0% (v/v) benzyl alcohol, pH 1.5–1.6.

Resin was ProSep-vA High Capacity media.

Aliquots were removed from each test solution at the required time point and spread-plated onto agar media (0.1 mL in duplicate). The limit of detection (LOD) is one colony forming unit (CFU) per agar plate or 10 CFU/mL. If an LRV is expressed in the Table as >, it means zero CFUs were detected on both duplicate agar plates when plating 0.1 mL of undiluted test solution, implying a complete kill. Such values were counted only if subsequent dilutions (10-fold, in series) were also free of any colonies.

*Bacteria mix included 1:1:1 ratio of *S. aureus*, *B. cepacia*, *P. aeruginosa*

A. niger spores were the most resistant to inactivation—twenty-four hour exposure to 2% benzyl alcohol resulting in <1 LRV. In contrast, PAB achieved a >3 LRV after only 2 hours. While 2% benzyl alcohol was more effective against

bacteria, achieving >2 LRV after 2 hours, PAB exhibited enhanced kill kinetics achieving >6 LRV in 2 hours.

Data from another experiment involving both naïve and process-challenged resin, as shown in Table 2, confirm the effectiveness of the PAB solution, achieving >3 LRV for *A. niger* spores and >5 LRV for other organisms within a 2 hour exposure.

Table 2. Microbial Kill (Log Reduction Values) with PAB in Presence of Naïve and Process-challenged Resin at 15 °C

Hours at 15 °C	LRVs Compared to PBS Control		
	1 hr	2 hr	3 hr
<i>Aspergillus niger</i> spores			
Naïve resin	2.58	3.23	ND
Process-challenged resin	2.73	3.23	ND
<i>Candida albicans</i>			
Naïve resin	3.22	>5.38	>5.43
Process-challenged resin	>5.38	>5.37	>5.39
Bacteria mix*			
Naïve resin	>7.11	>7.0	>7.03
Process-challenged resin	>7.11	>7.0	>7.03

ND = Not Determined

Process-challenged resin was subjected to feedstock and elution but not a low pH regeneration step, and transferred into an equilibration buffer for a “worst case” scenario.

PAB solution was 120 mM phosphoric acid, 167 mM acetic acid, and 2.0% (v/v) benzyl alcohol

Resin was ProSep-vA High Capacity media.

*Bacteria mix 1:1:1 ratio of *E.coli*, *S. aureus*, *B. cepacia*, and *P. aeruginosa*

Further experiments (not shown) demonstrated that testing a PAB -10% solution (108 mM phosphoric acid, 150 mM acetic acid, and 2% v/v benzyl alcohol) to account for “worst case” mixing tolerances at large scale, does not affect its ability to inhibit any of the microbes tested. In addition, aging the PAB solution for 23 days in the dark at room temperature (20–25 °C) in three container types (glass, stainless steel, HDPE bottles) did not hinder the microbial kill kinetics of all the organisms tested.

PAB VS. NAOH

As noted, NaOH cannot be used for sanitization of ProSep media, and has limitations in terms of concentration and contact time with other protein A affinity media. However, because NaOH is probably the most widely used sanitant within the bioprocessing industry, a comparison study on the effectiveness of PAB to NaOH was conducted. For this study, the microbial kill effectiveness of PAB was compared to that of two concentrations of NaOH (0.1 M and 0.5 M) in free solution.

The data in Table 3 again demonstrates that, as expected, microbial spores are more challenging than vegetative bacteria or yeast. PAB and 0.5 M NaOH demonstrate similar kill kinetics while 0.1 M NaOH was significantly less effective, even after several hours of exposure.

Table 3. Microbial Kill (Log Reduction Values) with PAB or NaOH in Free Solution (no resin) at 15 °C.

Hours at 15 °C	LRVs compared to PBS control				
	1 hr	2 hr	4 hr	6 hr	8 hr
<i>S. aureus</i>					
PAB	>7.44	>7.44	>7.44	>7.44	ND
0.5 M NaOH	>7.44	>7.44	>7.44	>7.44	ND
0.1 M NaOH	4.59	5.71	4.39	5.09	ND
<i>C. albicans</i>					
PAB	>5.81	>5.80	>5.80	>5.89	ND
0.5 M NaOH	>5.81	>5.80	>5.80	>5.89	ND
0.1 M NaOH	2.57	3.86	4.92	5.35	ND
<i>P. marquandii</i> spores					
PAB	ND	>5.02	>4.92	>4.85	>5.03
0.5 M NaOH	ND	>5.02	>4.92	>4.85	>5.03
0.1 M NaOH	ND	2.65	2.61	2.94	3.37
<i>A. niger</i> spores					
PAB	ND	>5.33	>5.48	>5.27	>5.19
0.5 M NaOH	ND	>5.33	>5.48	>5.27	>5.19
0.1 M NaOH	ND	0.53	0.71	0.76	0.85

PAB — RESIN STABILITY

Stability of the ProSep chromatography media to the PAB solution is as important as sanitization effectiveness. Initially, stability was determined by monitoring resin capacity over time during exposure to the PAB solution.

The dynamic capacity of the packed column (0.66 x 5.9 cm ProSep-vA High Capacity media) was first determined using human IgG and then the column was transferred into the PAB sanitant solution and stored. At the appropriate time, the column was washed with PBS to remove the PAB sanitant and the dynamic capacity re-measured. The column was then transferred back into PAB and the cycle repeated.

The data in Table 4 indicates a slightly greater loss of capacity over a 28 day period in the PAB solution compared to the standard shipping and storage buffer (1% (v/v) benzyl alcohol and 0.1 M sodium acetate pH 5.2). While this indicates that PAB is not a suitable solution for long term storage, resin stability is sufficient for it to be used as a periodic sanitant.

For instance, the use of a 3-hour sanitant step every 5 cycles run out to a total of 300 column cycles represents a total exposure time of only 180 hours or 7.5 days.

Table 4. Packed Column Stability (Dynamic Binding Capacity) in PAB

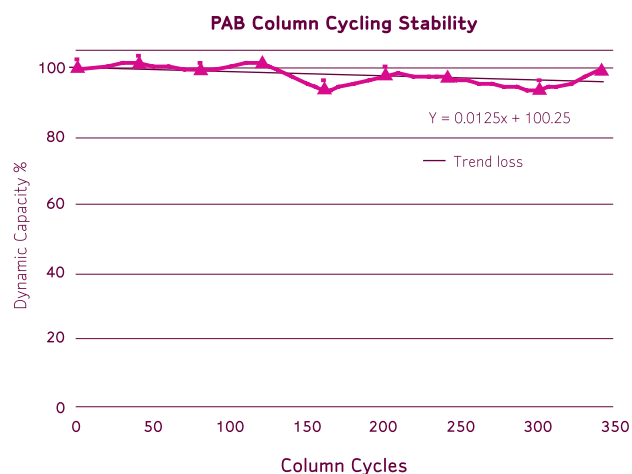
Conditions	Temp	Dynamic Capacity (%)				
		0 days	7 days	14 days	21 days	28 days
PAB	21 °C	100	97	93	94	86
0.1 M Ac 1% BA pH 5.2	21 °C	100	93	90	96	91

PAB solution was 120 mM phosphoric, 167 mM acetic acid and 2.2 % (v/v) benzyl alcohol.

Resin was ProSep-vA High Capacity media.

This situation was tested under simulated process conditions by running a packed column with buffers and monitoring column capacity over cycles. The results in Figure 1 show that over a total of 340 column cycles with a sanitization step every 5th cycle, dynamic capacity declined by only 4.25%, equivalent to a loss of 0.0125%/cycle, which is considered acceptable for process use.

Figure 1. Column Cycling Study — Dynamic Binding Capacity



2 mL (0.66 x 5.9 cm ProSep-vA High Capacity media) column.

Resin was ProSep-vA High Capacity media.

Each cycle comprised 10 CV PBS, 5 CV 0.1 M acetic acid pH 2.9 and 5 CV 150 mM phosphoric acid pH 1.5. After every 5th cycle, the column was exposed to 5 CV of a "worst case" PAB +10% solution (132 mM phosphoric acid, 184 mM acetic acid, and 2.42% benzyl alcohol) for a contact time of 3 hrs. Dynamic binding capacity with hlgG was determined every 40th cycle and compared to the zero time point capacity.

PAB — SANITANT ACCEPTABILITY

In addition to sanitization effectiveness and resin stability, other aspects to consider when selecting an appropriate sanitant solution include ease of detection, column washout and material compatibility.

As the pH of PAB is 1.6, change in pH can be readily used to monitor flush out from the column when re-equilibrating. Flush-out and re-equilibration is typically achieved in 2–3 column volumes depending on the strength of the re-equilibration buffer.

In general, PAB is compatible with commonly used column and system construction materials including stainless steel, polypropylene, PTFE, EPDM, glass and TPX® resin. The compatibility of acrylic to PAB is dependent on the grade of acrylic utilized. In 6-month continuous exposure tests, PAB constituents demonstrated compatibility with the acrylic used in Millipore's QuikScale® columns and current

engineered columns. However, other acrylic grades may not be compatible. If in doubt, it is recommended that users contact the appropriate equipment vendor for specific compatibility information.

APPLICATIONS SUPPORT

Should you have any questions relating to sanitizing ProSep protein A affinity media, or any other aspects of operation, Millipore's Chromatography Application Specialists are available to assist.

NOTE

Although ProSep-vA High Capacity media was used in these studies, PAB sanitant is equally applicable for use with ProSep-vA Ultra and ProSep Ultra Plus media.

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