Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems

Sodium hydroxide is widely accepted for cleaning and sanitization of chromatography resins and systems. The benefits of its use include efficacy, low cost, and ease of detection, removal, and disposal. As with any sanitizing agents, certain precautions should be taken and compatibility with both chromatography resins and systems determined.

This application note examines these aspects of using sodium hydroxide as a cleaning and sanitizing agent. Many examples of particular interest to producers of recombinant proteins, monoclonal antibodies, and oligonucleotides are included, as designing and scaling up validatable cleaning processes are critical issues in the commercial manufacture of these products (1).

Efficacy

Sodium hydroxide has shown to be effective in removing proteins and nucleic acids as well as in inactivating most viruses, bacteria, yeasts, fungi, and endotoxins. It is common practice in industrial manufacturing to save time by adding a salt, such as sodium chloride, to the sodium hydroxide solution to combine cleaning with sanitization.

Removal of proteins and nucleic acids

As a cleaning agent, sodium hydroxide saponifies fats and dissolves proteins (2). In general, sodium hydroxide can solubilize precipitated proteins and its hydrolyzing power is enhanced in the presence of chlorine (3).

The ability of sodium hydroxide to remove proteins and nucleic acids from chromatography resins depends on the nature of the resin, the sample, and sample impurities that might interfere with the cleaning efficiency. For example, a higher concentration of sodium hydroxide can be required if lipids are bound to a protein. To demonstrate the effectiveness of the sodium hydroxide solutions, users should periodically sample the stored solutions and also run blank gradients after cleaning (4).

Proteins

Sodium hydroxide has been used extensively to remove proteins from ion exchange, hydrophobic interaction, and gel filtration resins. Traditionally, the use of sodium hydroxide with affinity chromatography resins has been restricted because of the limited stability of most immobilized ligands. Today, recent developments have changed that picture and modern protein A-based affinity resins, such as MabSelect™ PrismA for monoclonal antibody purification, have significantly improved compatibility with sodium hydroxide. MabSelect PrismA with its alkali-stabilized protein A-derived ligand, was designed to withstand the use of up to 1.0 M sodium hydroxide solution as cleaning agent.

Nucleic acids

Nucleic acids can bind strongly to anion exchangers. However, work in our laboratories has shown that a combination of 1 M sodium hydroxide and 3 M sodium chloride, with a total contact time of one hour, effectively removes radiolabelled calf thymus DNA from DEAE Sepharose™ Fast Flow, a weak anion exchanger. A small percentage of the radiolabelled DNA was retained, and could not be eluted under any conditions tested. Others have found that lower concentrations or shorter contact times are insufficient to remove nucleic acid from DEAE Sepharose Fast Flow and restore the resin's separation capabilities.

Further work on Q Sepharose Fast Flow, a strong anion exchanger, has shown that 1 M sodium hydroxide combined with 1 M sodium chloride effectively removes DNA, but that the level of removal is dependent of the nature of the sample (5). The contact time for cleaning-in-place (CIP) in these experiments was two hours. For one sample, DNase was required to completely remove DNA from the anion exchanger.

Inactivation of viruses, bacteria, yeast, fungi, prions, endotoxins, and exotoxins

Viruses and prions

Experiments performed by a testing laboratory showed that 0.1 M sodium hydroxide was sufficient to inactivate the murine leukemia virus, a commonly used model enveloped virus (6). More recently, Q-One Biotech Ltd. has made available its data on the ability of sodium hydroxide to inactivate eight different viruses. Both 0.1 M and 0.5 M sodium hydroxide were tested and the kinetics of inactivation was reported (Table 1). It is worth noting that even highly resistant, nonenveloped viruses, such as canine parvovirus and SV-40, were inactivated by sodium hydroxide.

Furthermore, Creutzfeldt-Jakob disease (CJD), and its link to bovine spongiform encephalopathy (BSE), has raised further concerns about adventitious agents. Sodium hydroxide has shown to be effective in inactivating the BSE agent, which is otherwise extraordinarily resistant to most treatments, including ashing at 360°C for one hour (7, 8).

Bacteria, yeast, and fungi

Large amounts of microorganisms such as yeast and bacteria can destroy the function of chromatography columns and resins. These organisms can also have indirect effects, such as clogging of filters and other system components as well as produce harmful substances such as endotoxins, enterotoxins, and proteases. Table 2 shows that sodium hydroxide is effective in inactivating a number of yeasts and bacteria and that this inactivation is dependent of concentration, contact time, and temperature.

It is clear from Table 2 that bacterial spores might not be totally inactivated by sodium hydroxide. However, good manufacturing practice (GMP) and stringent hygienic routines should eliminate bacterial contamination in a manufacturing environment.

Table 2. Inactivation of microorganisms by NaOH

A) Types of microorganisms tested and their American Type Culture Collection (ATCC) number

Microorganism	ATCC No.	Type
Escherichia coli	8739	bacterium Gram –
Staphylococcus aureus	6538	bacterium Gram +
Pseudomonas aeruginosa	9027	bacterium Gram –
Bacillus subtilis	6633	spore-forming bacterium
Candida albicans	10231	yeast
Aspergillus niger	16404	mould

B) Test results at different times and temperatures

Organism	NaOH (M)	Time*	Temp. (°C)
E. coli	0.01	2 h	4 or 22
S. aureus	0.1	1 h	4 or 22
C. albicans	0.5	1 h	4 or 22
A. niger	0.5	1 h	4 or 22
B. subtilis spores	1.0	48 h†	22
B. subtilis spores	1.0	8 d‡	4
P. aeruginosa	0.5	1 h	22

* For reduction to below detection limit of < 3 organisms/mL.
† For reduction to below detection limit of 10 organisms/mL.
‡ For reduction to below detection limit of 100 organisms/mL.

To increase effectiveness against more resistant microorganisms, such as spore-forming Bacillus subtilis, the antimicrobial action of sodium hydroxide can be enhanced by the addition of ethanol. Table 3 illustrates the increased antimicrobial effect after addition of 20% ethanol to 0.1 and 0.5 M sodium hydroxide.

Table 1. The inactivation of eight different viruses* in 0.1 M and 0.5 M sodium hydroxide (italicized titer values indicate that no virus was detected in the sample and the values listed are theoretical minimum detectable titers)

Virus titers expressed in tissue culture infective dose (TCID50) units for all viruses except BHV and MLV, which are expressed in plaque forming units (pfu).

		HIV	BVDV	CPV	BHV	POL	SV-40	MLV	ADV
0.1 M NaOH	Spike	2.0×10^6	9.5×10^6	2.0×10^9	6.9×10^9	7.1×10^8	1.7×10^8	2.6×10^5	2.2×10^8
	t = 0 min	5.9×10^2	2.7×10^7	1.9×10^3	1.2×10^2	3.5×10^4	1.5×10^5	3.7×10^1	1.7×10^2
	t = 10 min	5.7×10^2	2.7×10^5	2.4×10^3	1.5×10^1	2.7×10^3	3.6×10^5	3.8×10^1	6.0×10^1
	t = 20 min	5.8×10^2	1.5×10^4	9.6×10^2	4.5×10^1	2.0×10^4	4.7×10^4	4.0×10^1	6.3×10^1
	t = 60 min	5.8×10^2	2.7×10^4	5.0×10^3	4.5×10^1	2.1×10^3	2.0×10^4	4.3×10^1	2.9×10^1
	Inactivation ($\log_{10}$)	> 3.5	2.5	5.6	8.2	5.5	3.9	> 3.8	> 6.9
0.5 M NaOH	Spike	2.0×10^6	9.5×10^6	2.0×10^9	6.9×10^9	7.1×10^8	1.7×10^8	2.6×10^5	2.2×10^8
	t = 0 min	5.7×10^2	1.9×10^4	9.4×10^2	5.9×10^1	1.1×10^5	1.5×10^5	6.3×10^1	9.4×10^1
	t = 10 min	5.6×10^2	1.3×10^2	1.2×10^3	5.9×10^1	1.1×10^5	1.7×10^3	4.7×10^1	7.5×10^1
	t = 20 min	5.6×10^2	1.7×10^2	1.5×10^3	5.9×10^1	2.0×10^4	8.4×10^3	4.7×10^1	2.0×10^1
	t = 60 min	6.7×10^2	1.7×10^2	1.5×10^3	5.9×10^1	6.2×10^3	1.0×10^2	5.5×10^1	2.2×10^1
	Inactivation ($\log_{10}$)	> 3.5	> 4.7	6.1	> 8.1	5.1	6.2	> 3.7	> 7.0

* HIV: human immunodeficiency virus type 1; BVDV: bovine viral diarrhea virus; CPV: canine parvovirus; BHV: bovine herpes virus type 1; POL: human poliovirus type 2; SV-40: simian virus-40; MLV: murine leukemia virus; ADV: human adenovirus type 2. Data from Q-One Biotech Ltd., Todd Campus, West of Scotland Science Park, Glasgow G20 0XA, Scotland.

Table 3. Antimicrobial effect (log10 reduction) of sodium hydroxide with the addition of 20% ethanol on Bacillus subtilis spores

Time	24 h	300 h
0.5 M NaOH	3 log	–
0.5 M NaOH with 20% ethanol	7 log	–
0.1 M NaOH	–	2 log
0.1 M NaOH with 20% ethanol	–	4 log

Microbial challenge tests are performed as part of a continuing effort to design systems and columns that meet sanitary design requirements. ÄKTAprocess™ is an automated chromatography system built for process scale-up and large-scale biopharmaceutical manufacturing (9). The ÄKTAprocess system has a number of features that make sanitization with 1 M sodium hydroxide simple and effective. Sanitization of ÄKTA™ pilot 600 chromatography system, used in process development, scale-up and scale-down, as well as in small-scale production, has also been performed using 1 M sodium hydroxide. The sanitization procedure showed excellent efficiency with at least 10^6 reduction in colony forming units for the four test organisms (10). Similarly, we have shown that sodium hydroxide is efficient as a CIP reagent for BPG columns (11), Chromaflow™ columns (12) and AxiChrom™ columns (13). The columns were subjected to different microbial strains and the specified CIP procedures using sodium hydroxide were proven to be very effective.

Endotoxins

Figure 1 illustrates the effectiveness of sodium hydroxide in inactivating a high load of endotoxins in solution. Note that much longer contact time is required when 0.1 M sodium hydroxide is used compared with a concentration of 0.5 or 1.0 M.

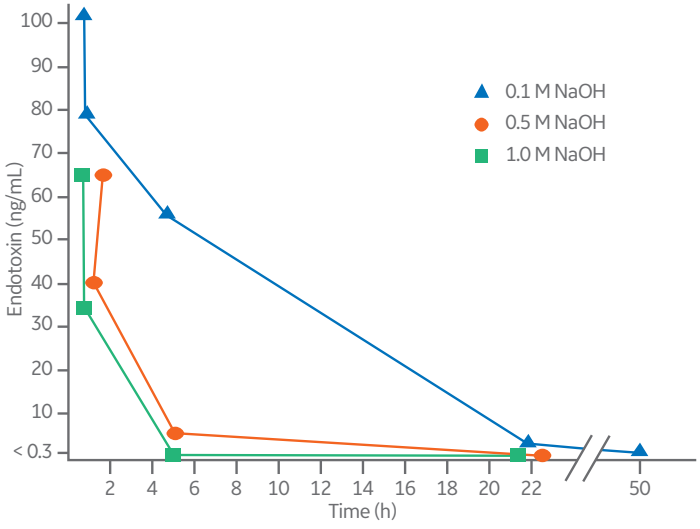

Fig 1. Inactivation of endotoxin by NaOH.

AxiChrom columns were tested for the efficiency of endotoxin clearance by challenge testing with 1 M sodium hydroxide (13). The columns were packed with Sepharose 6 Fast Flow resin and were challenged and incubated for 16 to 20 h at room temperature before being treated with 1 M sodium hydroxide and finally sampled. The results showed that treatment with 1 M sodium hydroxide effectively reduced the levels of endotoxin in the challenged column. A 6-log reduction of the endotoxin concentration was achieved and the final level in the column flowthrough was less than 0.05 EU/mL, which is below the USP recommendation for water for injection (WFI). These studies confirm that 1 M sodium hydroxide is an effective cleaning agent and also show that the AxiChrom column design allows operation at the high standards of hygiene required by GMP.

It is important to recognize that each feed stream is unique, and there might be a protection effect provided by other feed stream substances such as lipids and proteins. We therefore recommend endotoxin testing to be part of a routine evaluation of the effectiveness of a depyrogenation regime.

Exotoxins

Some bacterial strains produce exotoxins that are of concern to humans. Bacillus cereus, for example, produces cereulide, a heat-stable peptide-based exotoxin that causes nausea and vomiting when ingested. Once spores germinate and start to grow, exotoxin can be produced and secreted under the appropriate environmental conditions.

It has been demonstrated that clearance of the cereulide from mAb produced in Chinese hamster ovary (CHO) cells can be performed in a two-step chromatography process (14), where the bulk of the cereulide content is removed in the initial MabSelect SuRe capture step. After a known exposure to B. cereus cereulide, it is recommended to clean the protein A column using a stringent CIP protocol including, for example, 0.5 to 1.0 M NaOH as cleaning agent.

Other benefits of sodium hydroxide

Compared with other sanitizing agents, sodium hydroxide is inexpensive. Sodium hydroxide is also recommended for storage. According to the PDA Biotechnology Cleaning Validation Committee, concentrations of 0.1 to 1.0 M sodium hydroxide are common for storing packed chromatography columns (3). Removal of sodium hydroxide can be determined by simple in-line pH and conductivity measurements. Furthermore, the disposal of sodium hydroxide solutions is relatively straightforward, requiring no special measures.

Precautions

Always make sure that chromatography resins, columns, systems, and auxiliary components are compatible with sodium hydroxide at the concentration, time, and temperatures used. Also keep in mind that sodium hydroxide can be corrosive to both metal and skin (2). Please, read the material safety data sheet (MSDS) for sodium hydroxide before handling.

Compatibility

The concentration of sodium hydroxide employed for CIP and/or sanitization will often depend on the level of contamination. For chromatography resins, the ability to withstand stringent sanitizing conditions depends on the functional groups, attachment chemistries, and the stability of base matrices to alkaline conditions. This aspect of compatibility has been studied extensively and is well documented in the literature (15–19). Table 4 lists the general stability of a wide range of resins as a function of pH.

Table 5 illustrates the functional stability of Butyl Sepharose 4 Fast Flow after extended exposure to 1 M sodium hydroxide. There is no significant change in the retention time of four different standard proteins after four weeks storage of the resin in sodium hydroxide at room temperature.

Table 5. Functional stability of Butyl Sepharose 4 Fast Flow after CIP with NaOH (18)

Weeks in 1 M NaOH at room temperature	Retention time (min) A	B	C	D
0	8.50	23.80	37.40	51.65
3	8.40	23.08	37.13	51.48
4	8.44	23.26	36.84	51.47
Pooled S.D. (n = 9)	0.12	0.36	0.17	0.08

A: Cytochrome C; B: Ribonuclease A; C: Lysozyme; D: Chymotrypsinogen

Affinity ligands are in general more fragile and might not tolerate excessively harsh conditions and, consequently, the concentration of sodium hydroxide may have to be reduced. Nevertheless, recent developments have significantly improved the performance of affinity resins.

MabSelect SuRe™, MabSelect SuRe LX, and MabSelect PrismA are based on rigid, high-flow agarose matrix with alkali-stabilized protein A-derived ligands. The ligands were developed by protein engineering of one of the IgG-binding domains of protein A. Particularly alkaline-sensitive amino acids were identified and substituted with more stable ones. MabSelect SuRe and MabSelect SuRe LX resins have been tested for up to 200 cycles of CIP using 0.1 M NaOH and also repeated CIP cycles with 0.5 M NaOH (19). Figure 2 shows dynamic binding capacity (DBC) of MabSelect SuRe resin for polyclonal human IgG as a function of exposure to alkaline conditions. MabSelect resin, with a conventional recombinant protein A ligand, was used for comparison. At least 85% to 90% of the initial DBC of the MabSelect SuRe resin is retained after numerous CIP cycles with sodium hydroxide.

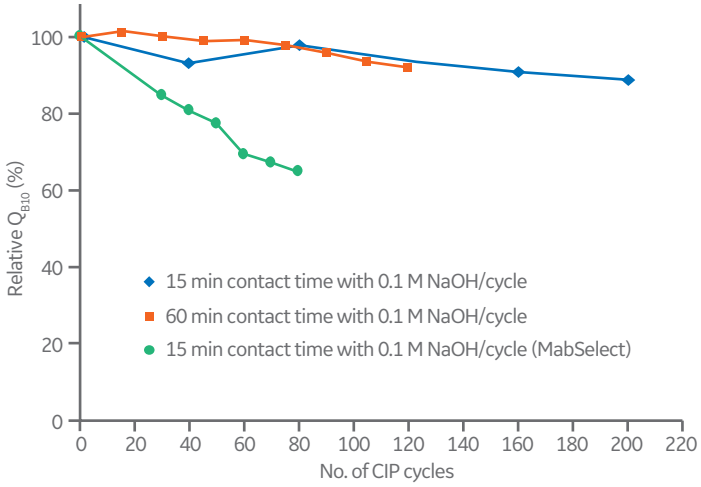

Fig 2. Dynamic binding capacity of MabSelect SuRe and MabSelect resins for polyclonal human IgG after CIP with 0.1 or 0.5 M NaOH for up to 200 cycles.

The enhanced alkaline stability of MabSelect PrismA enables efficient cleaning of the resin using up to 1.0 M NaOH over many purification cycles. Figure 3A shows relative remaining DBC of MabSelect PrismA as compared with MabSelect SuRe and MabSelect SuRe LX over repeated CIP cycles using 0.5 M NaOH as cleaning agent. As shown, MabSelect PrismA retains more than 93% of its initial DBC after 300 cycles. As shown in Figure 3B, MabSelect PrismA retains 90% of its initial DBC after 150 cycles with 1.0 M NaOH, while only 50% of the initial DBC of MabSelect SuRe LX remains after an equivalent number of CIP cycles.

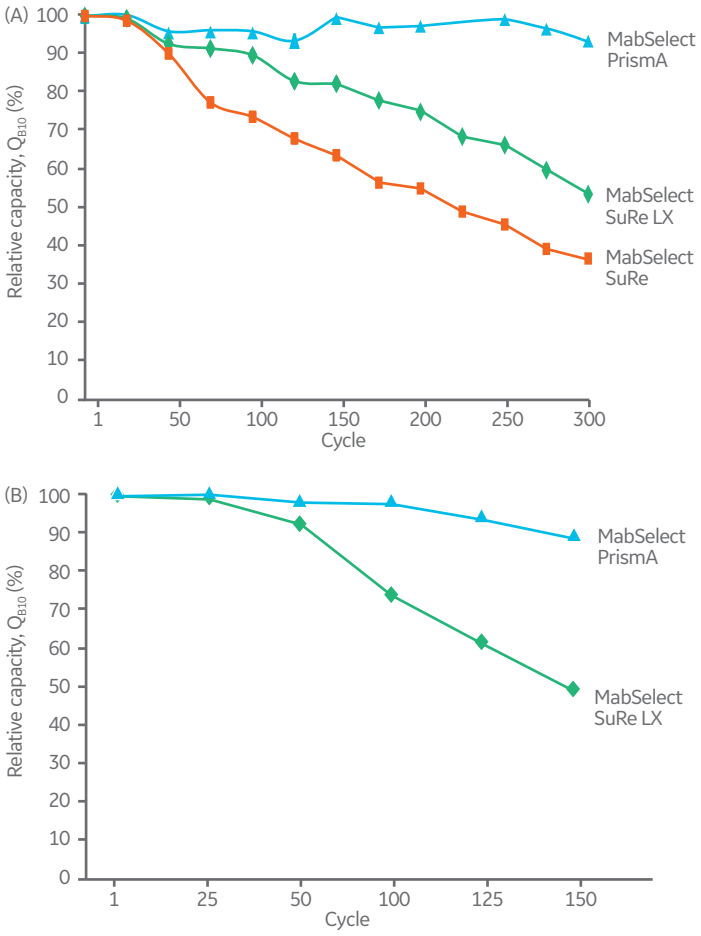

Fig 3. Relative remaining capacity (Q_{B10}) in 300 cycles, including CIP with (A) 0.5 M NaOH for 15 min/cycle or (B) in 150 cycles, including CIP with 1.0 M NaOH for 15 min/cycle.

Other affinity resins, such as Heparin Sepharose 6 Fast Flow, also tolerate sodium hydroxide (20). Figure 4 shows that Heparin Sepharose 6 Fast Flow withstands exposure to 0.1 M sodium hydroxide for long periods with no loss of binding capacity for antithrombin III (AT III). When contamination is severe, 0.5 M sodium hydroxide can be used effectively over shorter periods. However, a deterioration of the function will be seen over time, as is also shown in Figure 4.

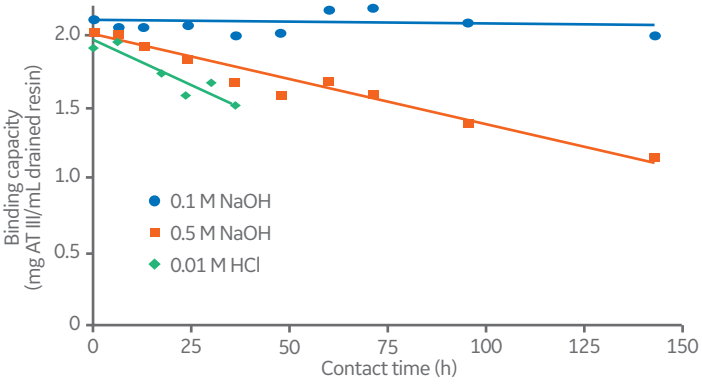

Fig 4. Functional stability of Heparin Sepharose 6 Fast Flow after CIP with 0.1 M and 0.5 M NaOH and 0.01 M HCl.

Storage

Most chromatography resins are delivered in 20% ethanol or optionally, 2% benzyl alcohol (including 0.2 M sodium acetate for some cation exchangers). Shelf life studies are performed on unused resins. GE Healthcare cannot define a storage time for resins that have been used and therefore recommend users to perform their own binding capacity test or other relevant test after storage according to their own protocol.

Table 4. Working and CIP pH stability ranges and storage solutions for various chromatography resins

	Operational pH	CIP pH	Storage	Alternative storage
Size exclusion chromatography resins				
Sephadex™ G-25	2–13	2–13	20% ethanol	0.01 M NaOH
Sephacryl™ High Resolution	3–11	2–13	20% ethanol	-
Superdex™ prep grade	3–12	2–14	20% ethanol	-
Sepharose Fast Flow	3–13	2–14	20% ethanol	0.01 M NaOH
Ion exchange chromatography resins				
Capto™ DEAE	2–9	2–14	20% ethanol	0.01 M NaOH
Capto Q	2–12	2–14	20% ethanol	0.01 M NaOH
Capto S	4–12	3–14	20% ethanol + 0.2 M NaAc	0.01 M NaOH
Capto S ImpAct	4–12	3–14	20% ethanol + 0.2 M NaAc	0.01 M NaOH
Capto Q ImpRes	2–12	2–14	20% ethanol	0.01 M NaOH
Capto SP ImpRes	4–12	3–14	20% ethanol + 0.2 M NaAc	0.01 M NaOH
MacroCap™ SP	3–12	2–13	20% ethanol + 0.2 M NaAc	-
DEAE Sepharose Fast Flow	2–9	2–14	20% ethanol	0.01 M NaOH
CM Sepharose Fast Flow	6–10	2–14	20% ethanol	0.01 M NaOH, 2% benzyl alcohol
SP Sepharose Fast Flow	4–13	3–14	20% ethanol + 0.2 M NaAc	0.01 M NaOH, 2% benzyl alcohol + 0.2 M NaAc
Q Sepharose Fast Flow	2–12	2–14	20% ethanol	0.01 M NaOH, 2% benzyl alcohol
ANX Sepharose 4 Fast Flow (high sub)	2–9	2–14	20% ethanol	0.01 M NaOH
ANX Sepharose 4 Fast Flow (low sub)	2–9	2–14	20% ethanol	0.01 M NaOH
SP Sepharose High Performance	4–13	3–14	20% ethanol + 0.2 M NaAc	0.01 M NaOH
Q Sepharose High Performance	2–12	2–14	20% ethanol	0.01 M NaOH
SOURCE™ 15S	2–13	1–14	20% ethanol + 0.2 M NaAc	0.01 M NaOH
SOURCE 30S	2–13	1–14	20% ethanol + 0.2 M NaAc	0.01 M NaOH
SOURCE 15Q	2–12	1–14	20% ethanol	-
SOURCE 30Q	2–12	1–14	20% ethanol	-
Q Sepharose XL	2–12	2–14	20% ethanol	0.01 M NaOH
SP Sepharose XL	4–13	3–14	20% ethanol + 0.2 M NaAc	0.01 M NaOH
SP Sepharose Big Beads	4–13	3–14	20% ethanol + 0.2 M NaAc	0.01 M NaOH
SP Sepharose Big Beads Food Grade	4–13	3–14	20% ethanol + 0.2 M NaAc	0.01 M NaOH
Q Sepharose Big Beads	2–12	2–14	20% ethanol	0.01 M NaOH

Multimodal chromatography resins				
Capto adhere	3–12	2–14	20% ethanol	0.01 M NaOH
Capto MMC	3–12	2–14	20% ethanol	-
Capto adhere ImpRes	3–12	2–14	20% ethanol	0.01 M NaOH
Capto MMC ImpRes	3–12	2–14	20% ethanol + 0.2 M NaAc	-
Capto Core 700	3–13	2–14	20% ethanol	-
PlasmidSelect Xtra	3–11	2–13	20% ethanol	-
Hydrophobic interaction chromatography resins				
Capto Butyl	3–13	2–14	20% ethanol	0.01 M NaOH
Capto Octyl	3–13	2–14	20% ethanol	0.01 M NaOH
Capto Phenyl (high sub)	3–13	2–14	20% ethanol	0.01 M NaOH
Capto Phenyl ImpRes	3–13	2–14	20% ethanol	0.01 M NaOH
Capto Butyl ImpRes	3–13	2–14	20% ethanol	0.01 M NaOH
Phenyl Sepharose Fast Flow (high sub)	3–13	2–14	20% ethanol	0.01 M NaOH
Phenyl Sepharose Fast Flow (low sub)	3–13	2–14	20% ethanol	0.01 M NaOH
Phenyl Sepharose High Performance	3–13	2–14	20% ethanol	0.01 M NaOH
Butyl-S Sepharose 6 Fast Flow	3–13	2–14	20% ethanol	0.01 M NaOH
Butyl Sepharose Fast Flow	3–13	2–14	20% ethanol	0.01 M NaOH
Butyl Sepharose High Performance	3–13	2–14	20% ethanol	0.01 M NaOH
Octyl Sepharose 4 Fast Flow	3–13	2–14	20% ethanol	0.01 M NaOH
Reversed phase chromatography resins				
SOURCE 15 RPC	1–12	1–14	20% ethanol	0.01 M NaOH
SOURCE 30 RPC	1–12	1–14	20% ethanol	0.01 M NaOH
Affinity chromatography resins				
Capto Blue	2–13	2–13	20% ethanol + K_2PO_4	0.01 M NaOH
Blue Sepharose 6 Fast Flow	4–12	3–13	20% ethanol + K_2PO_4	0.01 M NaOH
Chelating Sepharose Fast Flow	4–8.5*	2–14	20% ethanol	-
IMAC Sepharose 6 Fast Flow	4–8.5*	2–14	20% ethanol	-
Ni Sepharose 6 Fast Flow	4–8.5*	2–14	20% ethanol	-
Heparin Sepharose 6 Fast Flow	4–12	4–13	20% ethanol + 50 mM NaAc, pH 7.2	0.01 M NaOH
nProtein A Sepharose 4 Fast Flow	3†–9	3†–10	20% ethanol	-
MabSelect PrismA	3†–12	2†–14	20% ethanol	2% benzyl alcohol
MabSelect	3†–10	3†–12	20% ethanol	2% benzyl alcohol
MabSelect SuRe LX	3†–12	3†–13.7	20% ethanol	2% benzyl alcohol
MabSelect SuRe	3†–12	3†–13.7	20% ethanol	2% benzyl alcohol
MabSelect Xtra™	3†–10	3–12	20% ethanol	2% benzyl alcohol
rProtein A Sepharose Fast Flow	3†–10	3†–12	20% ethanol	-
Protein G Sepharose 4 Fast Flow	3†–9	2†–10	20% ethanol	-
rmp Protein A Sepharose Fast Flow	3†–10	3†–12	20% ethanol	-
Capto L	3–10	15 mM NaOH	20% ethanol	-

Operational pH: pH interval where the resin binds protein as intended or is needed for elution, without adverse long-term effect.

CIP pH: pH interval where the resin can be subjected to cleaning- or sanitization-in-place (accumulated 90–400 h at room temperature, both chromatography resin and feed dependent) without significant change in function (meaning will pass our QC test).

Storage: Recommended storage solution. Storage in ethanol or benzyl alcohol should be buffered for products containing S or SP ligands (0.2 M sodium acetate) and heparin ligands (50 mM sodium acetate, pH 7.2).

* Broken complex with metal ions.

† pH below 3 is sometimes required to elute strongly bound immunoglobulins. However, protein ligands might hydrolyze at low pH.

Note! In most cases, no long term stability data has been generated by GE Healthcare Life Sciences in 0.01 M NaOH. In some cases, accelerated studies at elevated temperature indicate that storage in 0.01 M NaOH can be a viable option but no guarantees can be made regarding retained function of the product.

The data presented here are an overview over recommended ranges. For more detailed information, please refer to the individual instructions, data files, and/or RSF files or contact our specialists in process chromatography or regulatory affairs.

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