Technical Note

Cleaning and Regeneration

of Fractogel® EMD sorbents

For chromatographic media a simple and efficient regeneration method is as important as high protein binding capacity and high resolution.
Regeneration should be performed directly in the column to avoid new packing. This method is called ”cleaning-in-place” (CIP).

The column geometry which provides a good distribution of cleaning solution is essential for complete removal of contamination. Especially in the region around the adaptor sealings, dead spaces have to be avoided because of their susceptibility to contamination with microrganisms.

When has a column to be cleaned?

Most important is the nature of the sample and the total amount of crude extract injected onto the column.
After running several cycles successively, the column should be regenerated with 1 to 2 M NaCl solution (ion-exchange chromatography) or with a buffer of low ionic strength (HIC, TAC) after each separation step.
In general, each column should be cleaned thoroughly after each separation step or at least after the experiments have been finished. Cleaning of the column has to be considered also when the gel bed is changing colours or the back pressure is increasing.

How to clean a column?

Various methods are feasible for cleaning chromatographic media. Chemical stability of the material and the type of contamination have to be taken into consideration.
In addition to organic solvents, bases and acids are being used. Polymeric matrices are characterized by higher chemical stability than inorganic sorbents based on silica gels (instable in presence of NaOH). They can also withstand treatment with acids in contrast to media based on carbonhydrates.

While lipids or similar substances (lipoproteins) can be removed with organic solvents like ethanol, isopropanol or ethylene glycol, denatured proteins can be effectively removed with sodium hydoxide (0.1 N up to 1 N NaOH).

If contaminants are tightly bound, it may be necessary to clean the column material with an acidic pepsin solution (0.1% pepsin in 0.01 N HCl), 6 M guanidine hydrochloride or diluted sodium lauroyl sarcosinsate solution (2% SLS in 0.25 M NaCl). Dilute the 20%-SLS-solution (cat.No. 7010) 1:10 to obtain the final concentration recommended for column cleaning. The removal of SLS is achieved by treatment with 20% 2-propanol in 0.01N HCl.

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Table: General aspects for column sanitization

	Equilibration	**Regeneration**	**CIP, cleaning-in-place**	**Sanitization**
When	after each run	after each run	depends on sample	batch
Goal	obtaining suitable starting conditions for next run	obtaining original state of chromatographic matrix in regard to function	removing contaminations without repacking column; avoids increasing impurities of desired protein, reduction of microorganisms	complete inactivation or removal of microorrganisms and cells
Method	4 column volumes of starting buffer	2 column volumes of high salt buffer containing 1-2 M NaCl	0.1-1 N NaOH, 20% isopropanol, 20% ethanol, 2% sodium lauroyl sarcosinate (SLS)	0.5-1 N NaOH or 2% SLS in 0.25 M NaCl, removal with 20% 2-propanol/0.01N HCl
Notes	pH, UV and conductivity of eluate have to be identical to starting buffer	removal of tightly bound contaminants (e.g. DNA from TMAE column)	reversing flow direction often advantageous	in case of NaOH longer contact time necessary, with SLS fast removal of contaminants

In case of anion exchangers with quaternary ammonium groups, application of 1 M NaOH should not be long-termed because it may cause successive cleavage of the functional groups. 1-2 column volumes can be applied without causing any problems.
When organic solvents are being used, the flow rate has to be maintained slow to avoid pressure build up (pressure limit: 5 bar). The same has to be considered for cleaning all Fractogel EMD materials with 20% acidic acid.

Table: Cleaning of Merck sorbents for Biochromatography

Sorbents	**NaCl**	**NaOH**	**HCl**	**0.01N HCl 0.1% Pepsin**	**2%SLS/ 0.25M NaCl**	**2-propanol**	**ACN**	**others / remarks**
Fractogel EMD ion exchanger	2 M	0,1-1N	1 - 2 N	+++	+++	20%	20%	20% ethanol, guanidine HCl, 6M urea
Fractogel EMD BioSEC	---	0,1-1 N	1 - 2 N	+++	+++	20%	20%	see ion exchanger
Fractogel EMD TA	---	0.1-1 N	1-2 N	+++	+++	20%	20%	50% ethylene glycol, guanidine HCl
Fractogel EMD Chelate	---	0.1-1 N	1-2 N	+++	n.d.	20%	20%	no EDTA
Fractogel EMD Propyl, Phenyl	---	0.1-1 N	1-2 N	+++	n.d.	20%	20%	50% ethylene glycol, guanidine HCl
Hydroxy apatite	1 M	0.1-0.5 N	>pH 6	---	---	100%	100%	no chelators

Abbreviations: SLS = sodium lauroyl sarcosinate.
ACN = acetonitrile

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