SAFC®
Pharma & Biopharma Raw Material Solutions

Application Note

Increase your Eshmuno® A resin lifetime with Ethylene Glycol Emprove® Evolve

Usage of Ethylene Glycol Emprove® Evolve and Eshmuno® A Chromatography Resin

Sodium hydroxide (NaOH) is a standard cleaning agent for protein A chromatography resins. Typically, a concentration of 0.1 mol/L NaOH is used. The inclusion of 20% ethylene glycol in a 0.1 mol/L NaOH cleaning solution increases the lifetime of Eshmuno® A columns significantly.

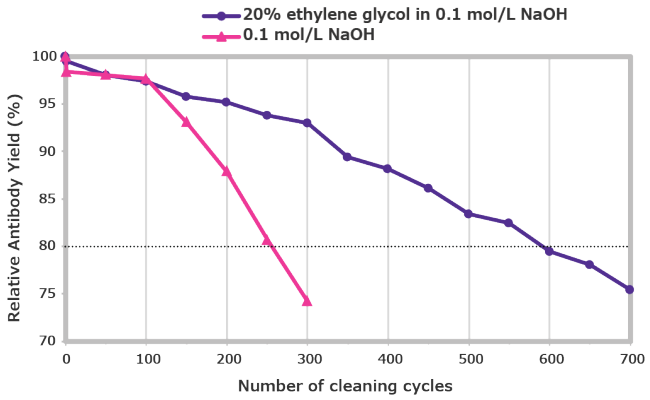

Figure 1: Relative antibody yields over 700 cleaning cycles of Eshmuno® A resin with and without inclusion of 20% ethylene glycol in a 0.1 mol/L NaOH cleaning solution.

Conclusion: The inclusion of 20% ethylene glycol in a 0.1 mol/L NaOH cleaning solution doubles the lifetime of the Eshmuno® A column.

Column lifetime is commonly determined by measuring the dynamic binding capacity (DBC). By examining the change in DBC over the course of multiple cleaning cycles, the impact of the cleaning procedure on column lifetime can be determined. A polyclonal antibody (Gammanorm®) was dissolved in buffer (25 mM Tris, 150 mM NaCl, pH 7.0) and the dynamic binding capacity of Eshmuno® A resin was measured. Up to 700 cleaning cycles were run with either 0.1 mol/L NaOH or 20% ethylene glycol in 0.1 mol/L NaOH with a contact time of 30 minutes per cycle. DBC was measured every 50 cleaning cycles. Figure 1 shows the relative antibody yield over the course of 700 cycles. The relative yield dropped below the acceptable limit of 80% after 250 cycles with the standard 0.1 mol/L NaOH solution, but with the inclusion of ethylene glycol, this limit was reached after 600 cleaning cycles, demonstrating that ethylene glycol increases the resin lifetime.

Ordering information

Description	Cat.No.
Ethylene glycol suitable for cleaning in place EMPROVE® EVOLVE	**1.37666**

Ordering information chromatography resin

Description	Cat.No.
Eshmuno® A chromatography resin	**1.20089**

MilliporeSigma

Removal of ethylene glycol from Eshmuno® A column

Before re-using the Eshmuno® A column after cleaning, the cleaning agent needs to be removed from the column by re-equilibrating the column with buffer. The column was re-equilibrated with 20 column volumes (CVs) of 20 mmol/L phosphate buffer (pH 7.0). The removal of 0.1 mol/L NaOH was monitored via pH measurement **(Table 1)**. To monitor the removal of ethylene glycol, fractions were collected and the concentration of ethylene glycol in the different fractions was determined with gas chromatography **(Table 1)**. pH 7.0 was reached after washing the column with 11.5 CVs of equilibration buffer. At the same time ethylene glycol concentration was below the limit of detection (LOD = 0.02 wt%).

Conclusion:

The inclusion of 20% ethylene glycol in a standard 0.1 mol/l NaOH cleaning solution does not impact re-equilibration time of the Eshmuno® A column. When pH 7.0 is reached both NaOH and ethylene glycol have been completely removed from the column.

Table 1: Changes in pH and ethylene glycol concentration over multiple column washes using 20 mmol/L phosphate equilibration buffer (pH 7.0).

Equilibration buffer volume (CV)	pH	Ethylene glycol concentration (wt%)
0.5	11.81	21.3
1.5	12.16	21.7
2.5	12.16	14.0
3.5	11.84	6.1
4.5	11.34	2.7
5.5	10.91	1.2
6.5	10.55	0.5
7.5	8.15	0.22
8.5	7.13	0.11
9.5	7.07	0.07
10.5	7.05	0.05
11.5	7.03	<LOD
12.5	7.03	<LOD
13.5	7.02	<LOD
14.5	7.01	<LOD
15.5	7.01	<LOD
16.5	7.00	<LOD
17.5	7.00	<LOD
18.5	7.00	<LOD
19.5	7.00	<LOD

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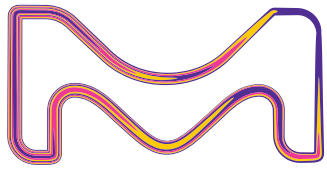

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