APPLICATION NOTE

Sodium Hydroxide (NaOH) Exposure Conditions for Successful Operation of GORE® Protein Capture Devices with Protein A for Drug Discovery Applications (PROA101 and PROA102)

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Objective

This application note provides data to support sodium hydroxide (NaOH) exposure limits for the 1.0mL and 3.5mL GORE Protein Capture Devices (PROA101 and PROA102). The cycling protocol utilizing 0.5 molar (M) NaOH supports 50 purification cycles with minimal impact to purification yield, device capacity, or assessed purity.

Materials/Equipment

- Liquid Chromatography System (LC System)
- 1.0mL GORE Protein Capture Device with Protein A (PROA101)
- Chemicals outlined in Table 1 protocols (see footnotes)
- CHO Cell Harvest: IgG1 monoclonal antibody (mAb) with a titer of 1.5 g/L

NaOH Stability Test

A 0.5M NaOH soak study was performed to evaluate dynamic binding capacity (DBC) degradation as a function of NaOH exposure time. Each exposure of NaOH was performed by loading 0.5M NaOH into the Device and holding for 0.5 hours. After each hold, a DBC test to 10% breakthrough ($DBC_{10\%}$) was performed to evaluate loss in binding capacity.

NaOH Cycling Study

The method outlined in Table 1 was used to perform 50 purification cycles of a mAb expressing CHO cell harvest with a titer of 1.5 g/L (mass loaded to 30mg per cycle). The method in Table 1 incorporates an acid strip step that replaces the normally recommended phosphate buffer solution (PBS) equilibration step to further reduce carry-over concerns from cycle to cycle.

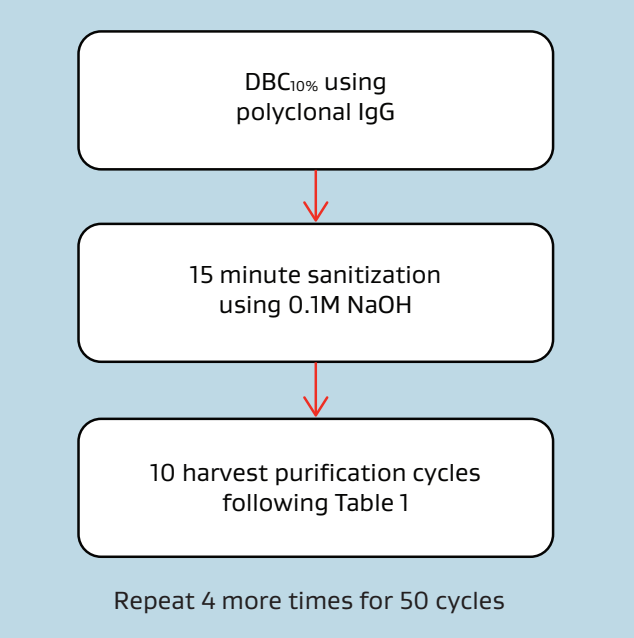
Table 1. Method Used to Perform 50 CHO Cell Harvest Purifications

Step	Solution	Column Volumes (CV)	Flow Rate (mL/min)	Time (min)
Equilibration	PBS*	5.0	3.0	1.7
Load	Harvest stock	n/a	3.0	5.2
High Salt Wash	PBS + 1M NaCl	6.0	3.0	2.0
Equilibration	PBS	6.0	3.0	2.0
Elution	Citrate **	4.0	3.0	1.3
Acid Strip	Citric Acid ***	3.0	3.0	1.0
CIP	0.5 M NaOH	3.6	1.2	3.0
Equilibration	PBS	6.0	3.0	2.0

* PBS phosphate-buffered saline (150 mM NaCl, 50 mM phosphate, pH 7.4)
** 100 mM Sodium Citrate dihydrate/Citric Acid pH 3.4
*** 100 mM Citric Acid pH 2.0

Figure 1 outlines the sequence of purifications which included (six) total DBC checks and a total of 50 purifications.

Figure 1. Sequence of Purifications for the PROA101 Device



Elution fractions were captured for cycles 2 through 5 and every 5 cycles. Yield, Host Cell Protein (HCP) contamination and leached Protein A were measured.

Results

NaOH Stability

The percent of DBC change observed across the NaOH soak study is described in Figure 2. To maintain the ability to load at 80% of the $DBC_{10\%}$, the recommendation is not to exceed 2.5 hours total contact time.

Figure 2. % DBC Change vs. 0.5M NaOH Exposure Time

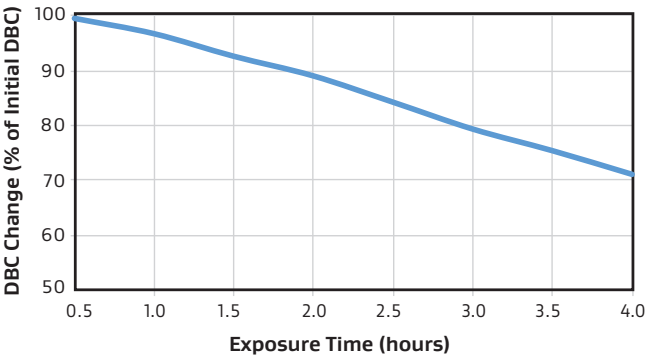
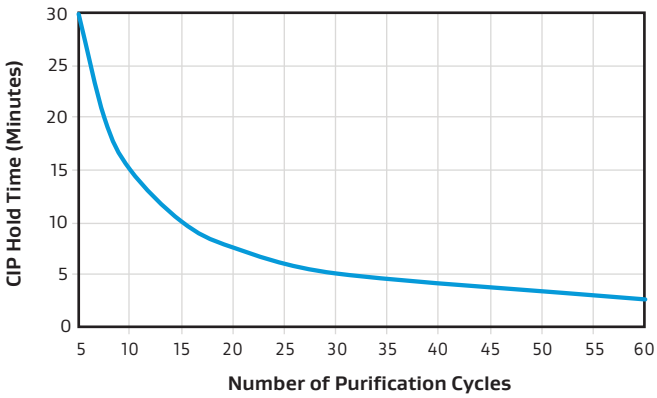


Figure 3 is an estimate of the number of purification cycles the PROA101 and PROA102 could tolerate for a given 0.5M NaOH CIP contact time, assuming a maximum Device contact time of 2.5 hours of 0.5M NaOH determined from the stability test.

Figure 3. Estimated Number of Purification Cycles based on Selected CIP Contact Time**



** Assuming 2.5 hour max exposure to 0.5M NaOH

Harvest Cycling

The method used in Table 1 demonstrated the successful completion of 50 purification cycles of a CHO cell harvest with a titer of 1.5 g/L loaded to 30mg per cycle. Figure 4 indicates the yield across 50 purification cycles. The yield was maintained at ≥ 90% for all purification cycles.

Figure 4. PROA101 mAb Yields across 50 Purification Cycles

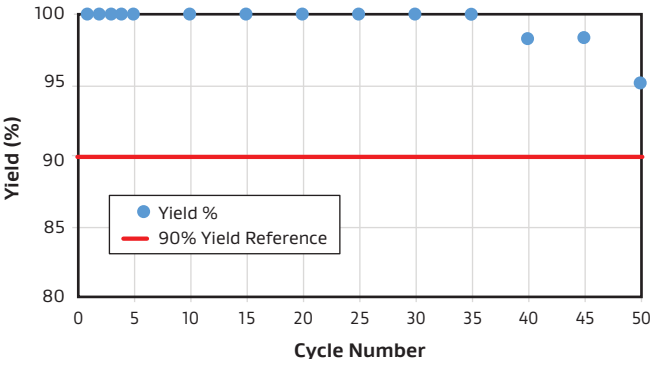


Figure 5 describes the Protein A leaching values and residual host cell protein (HCP) values observed across 50 purification cycles, following the method outlined in Table 1. Additionally, the Protein A leaching values were consistently below 10 ppm and the HCP remained consistent at approximately 2 Log Reduction Value (LRV).

Figure 5. HCP LRV & Leached Protein A through 50 Purification Cycles

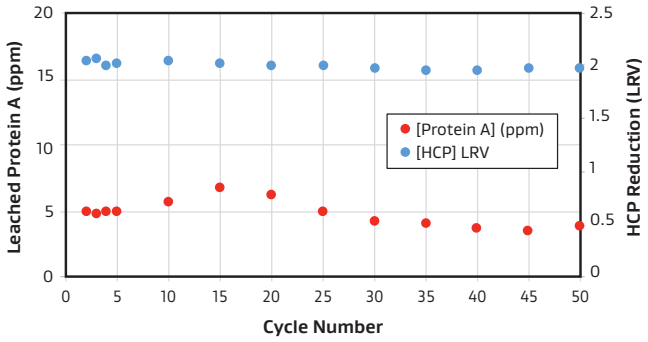
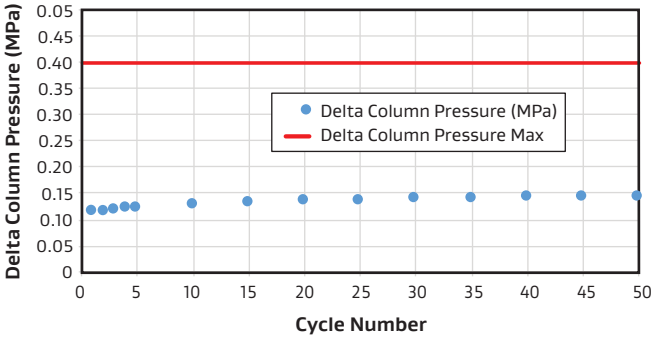


Figure 6 shows the delta column pressure observations across the 50 purification cycles following the method outlined in Table 1.

Figure 6. Delta Column Pressure through 50 Purification Cycles*



*Per GORE Protein Capture Device for Drug Discovery Operating Instructions (PB6576), the maximum operating delta column pressure is ≤ 0.40 MPa

Figure 7 depicts a representative purification cycle (Cycle 2) of the method outlined in Table 1, implementing a harvest load, high salt and PBS wash, elution, and an acid strip which directly transitions into the 0.5M NaOH CIP. No impact was observed when removing the PBS step between the acid strip and base CIP steps.

Figure 7. UV & pH Trace for Purification Cycle following Method in Table 1

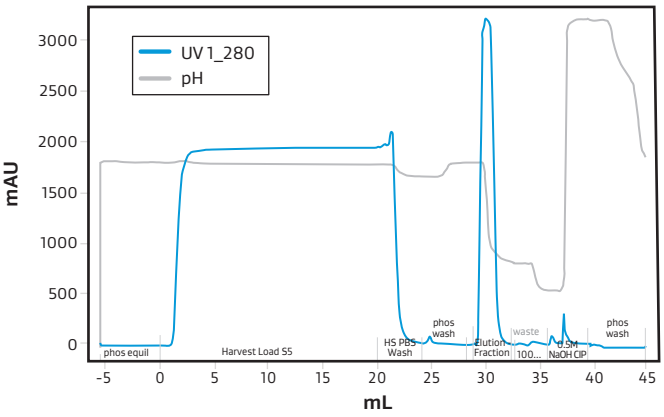


Table 2 shows the total mass processed by the PROA101 Device; the DBC before and after 50 purification cycles using 0.5M NaOH for three minutes of contact time per cycle; the average yield of the fractions collected throughout the 50 purification cycles; and the average protein A and HCP LRV through the 50 purification cycles.

Table 2. Purification Productivity and Performance over 50 Bind/Elute/CIP Cycles

Device	Total Mass Processed (g)	Device Size (mL)	Cycle 1 DBC (mg/mL)	Cycle 50 DBC (mg/mL)	Avg. Yield (%)	Avg. Leached Protein A (ppm)	Avg. HCP LRV
PROA101	1.5	1.0	≥ 30*	≥ 30*	99.4	4.7	2.0

*DBC evaluated at 20 seconds residence time

Conclusions

The purpose of this application note is to provide guidance for using 0.5M NaOH with the PROA101 and PROA102 Devices. The method provided demonstrates that up to 50 purification cycles can be performed with a three minute 0.5M NaOH contact time per cycle. The soak study enables customers to estimate the number of purification cycles that could be achieved based on 0.5M NaOH contact times for their specific CIP protocols. This protocol can enable yields that remain ≥ 90%, Device capacities ≥ 30 mg/mL, at acceptable purity values and it can be employed for both the 1.0mL PROA101 and the 3.5 mL PROA102 Devices.

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