TECHNICAL NOTE

Carryover Analysis of GORE Protein Capture Devices with Two Monoclonal Antibodies

Brad Joines, Mike McManaway and William Barrett, Ph.D., W. L. Gore & Associates, Inc.

Purpose

This technical note provides evidence based through ELISA assays that the GORE® Protein Capture Device did not have carryover after consecutive cycling of two different cell culture harvests when separated by a 15-minute sanitization cycle using 0.1 M NaOH.

Materials and Equipment

- Cytiva AKTA Pure 150 Liquid Chromatography System
- GORE Protein Capture Device, PROA101, 1 mL
- Chemicals outlined in Table 1:

Table 1. Chemicals utilized

Buffer/Solution	Composition
Tris Buffered Saline (TBS)	50mM Tris, 150 mM NaCl, pH 7.4
TBS + 1 M Sodium chloride (NaCl)	50mM Tris, 1.15 M NaCl, pH 7.4
Acetate	100 mM Acetate, pH 3.1
Acid Strip	100 mM Citric Acid, pH 2
Sodium hydroxide (NaOH)	0.5 M NaOH
20% Ethanol (EtOH)	20% EtOH/80% water (v/v)

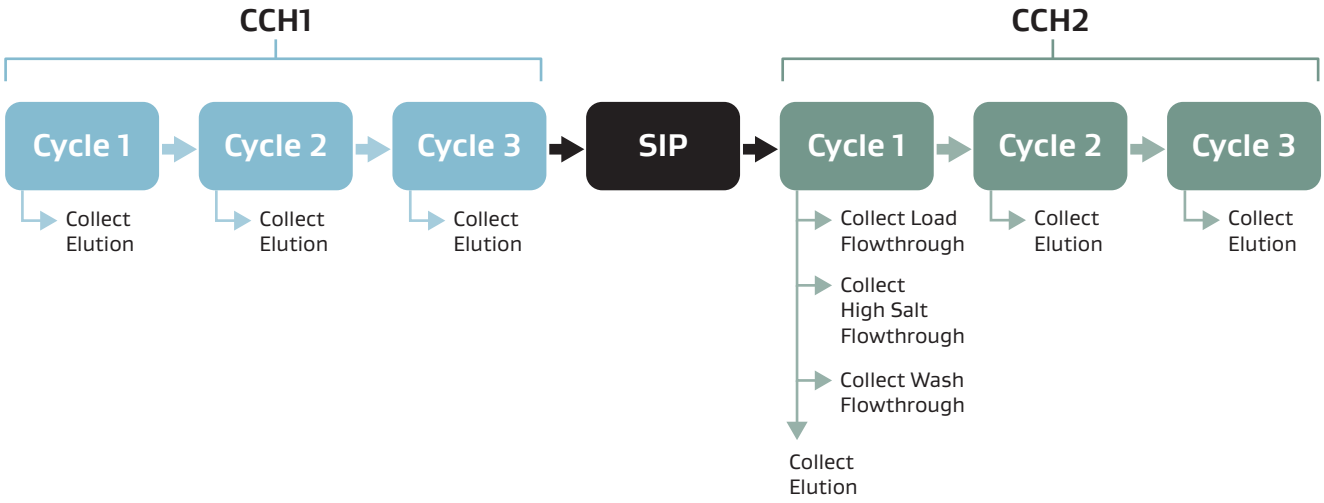
- Adalimumab Clarified CHO Harvest (CCH1) with a titer of 1.54 g/L
- Trastuzumab Clarified CHO Harvest (CCH2) with a titer of 4.12 g/L
- Adalimumab ELISA Kit (Part Number: ab237641, Abcam, Cambridge, UK)
- Anti-HER2 ELISA Kit (Part Number: ab237645, Abcam, Cambridge, UK)

Methods

Cycling

A standard cycling protocol was used for both CCH1 and CCH2 per Table 2. The load volume was adjusted for each harvest titer, loading to 24 mg based on 80% of $DBC_{10\%}$ at 20 seconds residence time (SRT) of 30 mg/ml. The fractionation sequence was setup as per Table 3. Multiple cycles of CCH1 were run followed by a 15-minute contact time sanitization with 0.1M NaOH was performed between prior to switching to CCH2. A rapid CIP method using 30 second contact time with 0.5 M NaOH was performed between each cycle for the CIP. The cycling was done to have multiple loads of antibody 1 (CCH1) followed by SIP, then multiple loads of antibody 2 (CCH2). Figure 1 represents the cycling approach for each antibody, and the fractions collected for analysis with both ELISA kits.

Figure 1. Graphical depiction of cycling study with collected fractions for ELISA analysis.



CCH cycling method

Table 2. CCH Cycling Protocol

Step	Solution/Buffer	Column Volumes (CV)	Flow Rate (mL/min)
Sample Load	CHO Harvest Load	TBD per CCH titer	3
Equilibration Wash	TBS	2	3
High Salt Wash	TBS + 1M NaCl	5	6
Equilibration Wash	TBS	5	6
Elution	100 mM Acetate	6, (fraction = 4.5)	6
Acid Strip	100 mM Citric Acid	3	6
Base Strip	0.5 M NaOH	3	6
Equilibration Wash	TBS	≈ 8.4, (conductivity <20 mS/cm & pH< 8) +/- 0.5, stable for 0.5 min	6

CCH cycling and fraction sequence

Table 3. CCH Cycling and fraction sequence

	CCH1 Fractionation			
	Load Flowthrough	High Salt Flowthrough	TBS Wash Flowthrough	Elution
Cycle 1	No	No	No	Yes
Cycle 2	No	No	No	Yes
Cycle 3	No	No	No	Yes
Sanitization 0.1 M NaOH at 15 minutes contact time				

	CCH2 Fractionation			
	Load Flowthrough	High Salt Flowthrough	TBS Wash Flowthrough	Elution
Cycle 1	Yes	Yes	Yes	Yes
Cycle 2	No	No	No	Yes
Cycle 3	No	No	No	Yes

ELISA Testing

The ELISA testing was performed using the Abcam Adalimumab ELISA Kit (ab237641) for Adalimumab detection and the Abcam Anti-HER2 ELISA Kit (ab237645) for Trastuzumab detection.

The ELISA testing was performed according to kit instructions. The test samples were diluted with PBS to fall within the standard curve provided in the ELISA kits.

To determine the presence of either monoclonal antibody within the test samples, the Adalimumab and Trastuzumab sets of samples were tested on both kits.

Results

ELISA Results

Adalimumab ELISA results for each fraction collected are shown in Table 4.
Trastuzumab(anti-HER2) ELISA results for each fraction collected are shown in Table 5.

Table 4. Results of Abcam Adalimumab ELISA Kit Testing

mAb	Sample	Adalimumab (ng/mL)
Trastuzumab (CCH2) Samples	Load Flowthrough	BDL
	High Salt Flowthrough	BDL
	TBS Wash Flowthrough	BDL
	Cycle 1 Elution	BDL
	Cycle 2 Elution	BDL
	Cycle 3 Elution	BDL
	Trastuzumab Feed Sample	BDL
Adalimumab (CCH1) Samples	Cycle 1 Elution	906995
	Cycle 2 Elution	1175390
	Cycle 3 Elution	1153015
	Adalimumab Feed Sample	332985

BDL – Below Detection Limit (~30ng/mL pre-dilution correction)

Table 5. Results of Abcam Anti-HER2 ELISA Kit Testing

mAb	Sample	Trastuzumab (ng/mL)
Trastuzumab (CCH2) Samples	Load Flowthrough	BDL
	High Salt Flowthrough	BDL
	TBS Wash Flowthrough	BDL
	Cycle 1 Elution	2945990
	Cycle 2 Elution	3042940
	Cycle 3 Elution	2910100
	Trastuzumab Feed Sample	ADL
Adalimumab (CCH1) Samples	Cycle 1 Elution	BDL
	Cycle 2 Elution	BDL
	Cycle 3 Elution	BDL
	Adalimumab Feed Sample	BDL

BDL – Below Detection Limit (~10ng/mL pre-dilution correction),
ADL – Above Detection Limit (~300ng/mL pre-dilution correction)

Conclusions

The expectation was that no adalimumab would be detected in the trastuzumab fractions and no trastuzumab would be detected in the adalimumab fraction. The results showed no detectable trastuzumab in adalimumab fraction, while the adalimumab elutions showed appropriate reactivity to the adalimumab kit. The trastuzumab flow-through and washes showed no detectable limit of adalimumab showing no carryover by ELISA, and no detectable trastuzumab indicating complete binding as detectable by ELISA. The elution fractions of trastuzumab showed appropriate reactivity to anti-HER2 kit and no detectable level of adalimumab. This data indicates that with a SIP using 0.1 M NaOH for 15 minutes between monoclonal antibodies in conjunction with a rapid CIP for 30 seconds using 0.5 M NaOH resulted in no carryover from one monoclonal antibody to another as detectable by ELISA assay.

Gore PharmBIO Products

Our technologies, capabilities, and competencies in fluoropolymer science are focused on satisfying the evolving product, regulatory, and quality needs of pharmaceutical and bioprocessing customers, and medical device manufacturers. GORE® Protein Capture Devices with Protein A, like all products in the Gore PharmBIO Products portfolio, are tested and manufactured under stringent quality systems. These high-performance products provide creative solutions to our customers' design, manufacturing, and performance-in-use needs

NOT INTENDED FOR USE in medical device or food contact applications or with radiation sterilization. Please see operating instructions for intended use.

All technical information and advice given here is based on our previous experiences and/or test results. We give this information to the best of our knowledge, but assume no legal responsibility. Customers are asked to check the suitability and usability of our products in the specific applications, since the performance of the product can only be judged when all necessary operating data is available. Gore's terms and conditions of sales apply to the purchase and sale of the product.

Americas | W. L. Gore & Associates, Inc.
402 Vieve's Way • Elkton, MD 21921 • USA
Phone: +1 410 506 1715 • Toll-free (US): 1 800 294 4673
Email: pharmbio@wlgore.com

Europe | W. L. Gore & Associates, GmbH
Wernher-von-Braun-Strasse 18 • 85640 Putzbrunn, Germany
Phone: +49 89 4612 3456 • Toll free: 0 800 4612 3456
Email: pharmbio_eu@wlgore.com

gore.com/pharmbio

PB12254-R1-TEC-US-DEC22