Fast track formulation pH screening with automated buffer exchange on Big Tuna

Introduction

The critical process of screening formulation buffers to optimize stability is labor intensive and time consuming, which are often limiting factors in biologics development. The conformational, chemical, and colloidal stability of a protein are strongly influenced by the buffer solution. Altering buffer salts, pH, ionic strength, excipients, and surfactants may increase or decrease the stability of a molecule. A common step in the process of optimizing buffer conditions for a biologic molecule is screening across a pH range.

Conventional exchange methods are prone to inconsistency and difficult to manage in larger numbers. Automated buffer exchange systems can provide a more uniform sample handling approach, reduce hands on time, and increase throughput. Automating a pH screen can further cut down the time required to optimize buffer conditions for new biologic molecules.

Big Tuna was developed to automate the buffer exchange process, enable increased throughput, and provide degrees of process control that are otherwise inaccessible by manual methods (Figure 1). Big Tuna uses a pressure-based ultrafiltration/diafiltration (UF/DF) method to remove buffer.

Figure 1: Big Tuna automates buffer exchange for up to 96 unique samples with Unfilter 96 or up to 24 unique samples with Unfilter 24.

During the pressure-based filtration, the plate is gently mixed, ensuring that protein cannot accumulate at the membrane surface, while keeping flow more uniform and faster than dead-end filtration methods. Big Tuna also enables concentration to a new target after the exchange is complete.

Buffer exchange with Big Tuna is highly customizable and adaptable, allowing for buffer exchange of up to 96 unique proteins and formulations in a single experiment. Unchained Labs developed two filter plate formats for

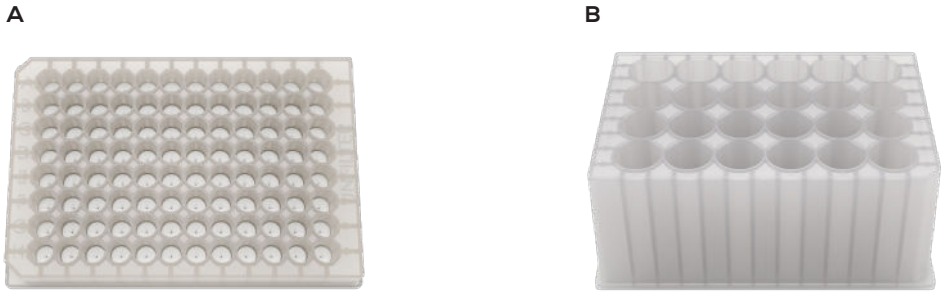

Figure 2: Big Tuna can accommodate both Unfilter 96 and Unfilter 24. **(A)** Unfilter 96 allows for up to 96 samples to be buffer exchanged simultaneously at volumes of 100-450 µL per well. **(B)** Unfilter 24 allows for up to 24 samples to be buffer exchanged simultaneously at volumes of 0.45-8 mL per well.

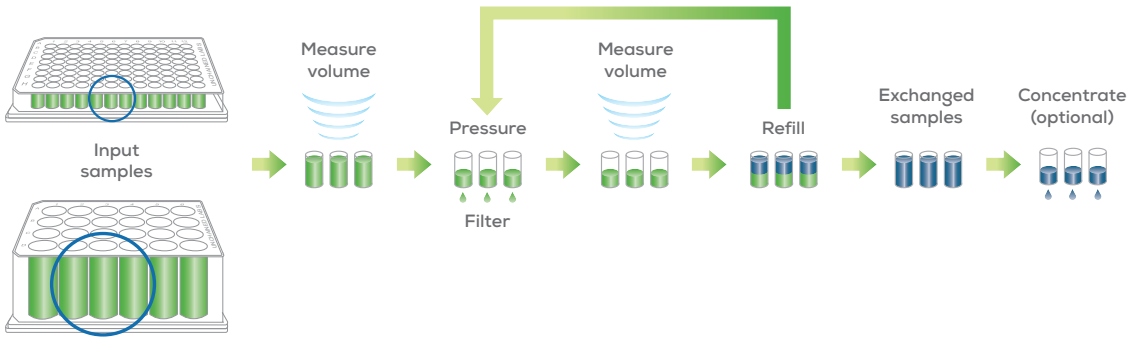

Figure 3: Big Tuna uses a pressure-based UF/DF method with gentle orbital mixing to buffer exchange proteins with the Unfilter 96 and Unfilter 24.

this process. The Unfilter 96 and Unfilter 24 are regenerated cellulose (RC) filtration plates designed to withstand 60 psi pressurization during the buffer exchange process (Figure 2). Unfilter 96 can process up to 96 samples ranging from 100-450 µL; Unfilter 24 can process 24 samples ranging from 0.45-8 mL in a single run. Before the run, the Unfilter 96 or Unfilter 24 is filled with the protein to be exchanged and placed in the exchange chamber. The new buffers are placed on the deck. During the run, Big Tuna alternates between filtration, volume measurement, and new buffer addition to buffer exchange proteins (Figure 3).

In this application note, we demonstrate that Big Tuna can be used to conduct a pH screen on a monoclonal antibody (mAb) in 20 mM histidine across a pH range of 6.3 – 7.4. Each of the 12 formulations were exchanged in duplicate at 4 mL per well in an Unfilter 24.

Methods

Protein and buffer preparation

A stock mAb was prepared at approximately 45 mg/mL in its proprietary stock buffer. 20 mM histidine was prepared across a pH range of 6.3 – 7.4, at increments of 0.1 pH units, totaling 12 buffer formulations. The buffers were placed on the deck of Big Tuna prior to buffer exchange.

The mAb was manually pipetted into 24 wells of a 10 kDa Unfilter 24 (4 mL/well), where it was exchanged into the 12 buffers prepared for the pH screen, resulting in 12 formulations consisting of a mAb in 20 mM histidine across a pH range of 6.3 – 7.4, each in duplicate.

Parameter	Setting
Target exchange percentage	96%
Target volume removed per cycle	33%
Initial concentration	45 mg/mL
Initial well volume	4 mL
Target final concentration	45 mg/mL
Target final well volume	4 mL

Table 1: Key buffer exchange parameters used for this experiment. Pressurization cycle duration was automatically adjusted to reach an average of 33% volume removed from any well. 12 conditions in duplicate were run in a single experiment.

Buffer exchange

Key buffer exchange parameters are outlined in Table 1. The buffer exchange protocol was set to 96% total exchange per pool with a target volume removal per cycle of 33%. To maintain the same concentration before and after buffer exchange, final well volume was targeted to 4 mL. Throughout automated buffer exchange, pressurization cycle duration was automatically adjusted by Big Tuna to have the average volume removed per well approximately equal to the user-defined target volume.

Big Tuna Client was used for experimental design and execution. Results were analyzed by exporting data to Excel directly from Big Tuna Client. Aver-

age cycle duration, initial and final well volumes, and final percent exchange were calculated in Excel.

Protein concentration

Stunner was used to analyze the concentration of the stock mAb and the 12 exchanged formulations. Protein concentration was determined using the E1% specific to the stock mAb. All measurements were done in triplicate and average concentration is reported in all cases.

Results

Stock protein formulations

The stock mAb was prepared in its stock buffer at a target concentration of approximately 45 mg/mL. Table 2 shows the actual mAb concentration before and after buffer exchange, and average final volume of each formulation.

Buffer exchange

The target percent exchange of 96% for each well was reached in 10 buffer exchange cycles that averaged 21 minutes per cycle (range: 19-22 minutes/cycle). The duration of each cycle adjusted automatically so the average volume removed from any well per cycle was approximately the target of 33% exchange per cycle. The total time to complete the buffer exchange for this pH screen on an Unfilter 24 was approximately 7.1 hours.

Initial fill volume per well was 4,000 µL. Final fill volume per well was ultrasonically measured as 4,004 ± 15 µL, at the target of 4,000 µL per well.

A target percent exchange of 96% was set per pool, so every pool must reach a minimum of 96% exchange. Because flow rate of solutions varied, some pools exchanged to >96% to ensure that all pools exchanged to at least 96%. The average percent exchange per pool across the Unfilter 24 was 97.9%, with a minimum percent exchange of 96.5% and a maximum of 99.1% (Table 2). Each of the 12 formulations did exchange at slightly different rates, but despite these differences buffer exchange was successful across the Unfilter 24 due to Big Tuna optimizing the pressurization cycle duration in real time.

Buffer target pH	Initial volume (µL)	Average final volume (µL)	Initial conc (mg/mL)	Average final conc (mg/mL)	Average % exchange
6.3	4,000	4,022	45.8	44.7	99.1
6.4	4,000	4,026	45.8	45.0	98.9
6.5	4,000	3,991	45.8	45.2	98.8
6.6	4,000	4,010	45.8	45.6	98.5
6.7	4,000	3,987	45.8	45.4	98.3
6.8	4,000	4,020	45.8	45.4	98.2
6.9	4,000	3,999	45.8	45.0	96.9
7.0	4,000	3,990	45.8	45.3	97.8
7.1	4,000	4,018	45.8	45.2	97.3
7.2	4,000	3,988	45.8	45.6	97.3
7.3	4,000	4,005	45.8	45.5	96.5
7.4	4,000	3,989	45.8	45.7	96.9
Average	**4,000**	**4,004 ± 15**	**45.8**	**45.3 ± 0.3**	**97.9**

Table 2: Protein concentrations of each formulation before and after buffer exchange as determined by Stunner. Final volumes for each formulation after buffer exchange as determined by the ultrasonic volume sensor on Big Tuna.

Final protein concentration

After buffer exchange on Big Tuna, the target final concentration was equal to the initial concentration, approximately 45 mg/mL. Table 2 shows the actual protein concentrations before and after buffer exchange as measured by Stunner. Actual final concentrations were approximately equal to the target 45 mg/mL. Final concentrations paired with final well volumes show that protein recovery was high for all wells, despite any variation in concentration.

Conclusion

Big Tuna is capable of conducting high-throughput pH screens with minimal hands on time. Big Tuna exchanged a mAb into 12 buffer formulations across a pH range of 6.3 – 7.4. An automated pH screen with Big Tuna allows researchers to make informed decisions to optimize biologic formulations with significantly less hands-on time.

The ability of Big Tuna to adjust pressurization cycle duration after each cycle provides efficiency and precise volume control during the exchange process. Initial and final protein conditions, such as well volume, concentration, and target percent exchange showed consistency across the Unfilter 24 despite differences in formulations.

Unchained Labs
6870 Koll Center Parkway
Pleasanton, CA 94566
Phone: 1.925.587.9800
Toll-free: 1.800.815.6384
Email: info@unchainedlabs.com

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