Automated platform buffer screening for multiple proteins on Big Tuna

Introduction

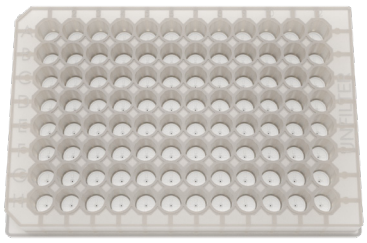
The critical process of screening formulation buffers to optimize stability is labor-intensive and time-consuming, which is often a limiting factor in biologics development. The conformational, chemical, and colloidal stability of a protein are strongly influenced by the buffer solution. Altering buffer salts, pH, ionic strength, excipients, and surfactants may increase or decrease the stability of a molecule. To alleviate some of the time requirements in developing a new biologic molecule, a platform buffer screen is typically used to screen common formulation conditions to quickly narrow down optimal buffer conditions. A platform buffer screen analyzes the stability of a new molecule with common buffers, excipients, and surfactants in common pH ranges.

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 and degrees of process control that are otherwise inaccessible by manual methods. Automating a platform buffer screen can further cut down the time required to optimize buffer conditions for new biologic molecules. Big Tuna automates the process, reducing hands-on time and enabling increased throughput.

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.

Big Tuna was developed to address gaps in low-volume, high-throughput buffer exchange (Figure 1). Big Tuna uses a pressure-based ultrafiltration/diafiltration (UF/DF) method to remove buffer. 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 automates the buffer exchange process, reduces hands-on time and increases throughput. Big Tuna also enables concentration to a new target after the exchange is complete.

A



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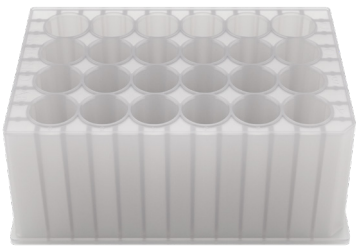

Figure 2: Big Tuna can accommodate both Unfilter 96 and Unfilter 24. **A:** Unfilter 96 allows for up to 96 samples to be buffer exchanged simultaneously at volumes of 100-450 µL per well. **B:** Unfilter 24 allows for up to 24 samples to be buffer exchanged simultaneously at volumes of 0.45-8 mL per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

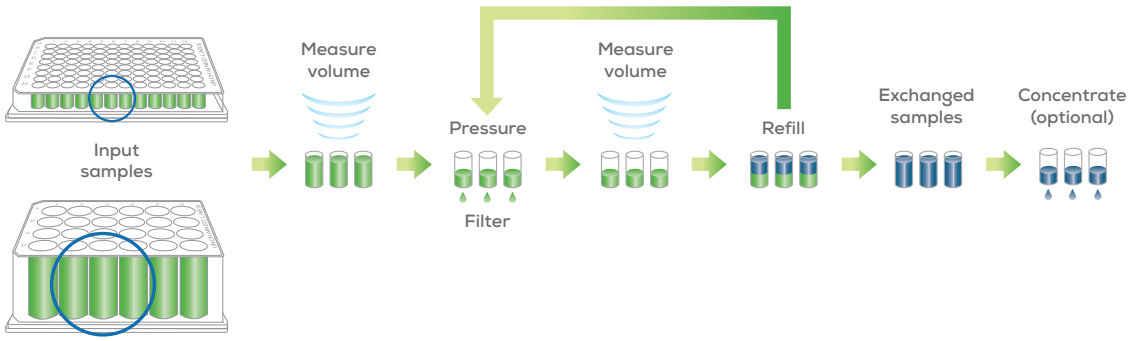

Figure 3: Big Tuna uses a pressure-based UF/DF method with gentle orbital mixing to buffer exchange proteins with the Unfilter 96 and Unfilter 24.

Buffer exchange with Big Tuna is highly customizable and adaptable, allowing for buffer exchange of up to 96 or 24 unique proteins and formulations in a single experiment. Unchained Labs developed two filter plate formats for this process. The Unfilter 96 and 24 are filtration plates designed to withstand 60 psi pressurization during 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 buffer is 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, Big Tuna is used to conduct a platform buffer screen on four monoclonal antibodies (mAbs) in six unique buffer formulations and to automatically concentrate the resulting formulations 5-fold. The platform buffer screen performed on Big Tuna consisted of a single base buffer with one of five excipients for each of the four mAbs. Solutions widely varied by flow rate, and Big Tuna monitored volume to ensure that no solutions dried out and everything reached the final concentration and exchange targets.

Methods

Protein and buffer preparation

Four stock mAbs (mAb A, mAb B, mAb C, and mAb D) were prepared at 10 mg/mL in their stock buffers. A base buffer of 10 mM histidine was prepared at pH 6.0 with one of five excipients: 150 mM NaCl, 150 mM sucrose, 75 mM mannitol, 150 mM arginine, 100 mM glycine, and no excipient control. Each of the 6 unique prepared buffers was manually pipetted into 2 mL 96-well plates and placed on the Big Tuna deck.

Each mAb was manually pipetted into 6 wells of a 10 kDa Unfilter 24 (8,000 µL/well), where they were exchanged into the 6 buffers prepared for the platform buffer screen. Buffer exchange resulted in 24 biologic formulations consisting of four mAbs each in six unique buffers (Table 1).

Buffer exchange

Key buffer exchange parameters are outlined in Table 2. The buffer exchange protocol was set to

Condition	Variable(s) tested
Buffer	10 mM histidine
pH	6.0
Excipients	None, 150 mM NaCl, 150 mM sucrose, 75 mM mannitol, 150 mM arginine, 100 mM glycine
Antibodies	mAb A, mAb B, mAb C, mAb D

Table 1: Formulation conditions studied on Big Tuna. A total of 24 conditions were run in a single experiment.

Parameter	Setting
Target exchange percentage	96%
Target volume removed per cycle	66%
Initial concentration	10 mg/mL
Initial well volume	8 mL
Target final concentration	50 mg/mL
Target final well volume	1.6 mL

Table 2: Key buffer exchange parameters were user defined in the Big Tuna software. Pressurization cycle duration was automatically adjusted to reach a maximum of 66% volume removed from any well.

96% total exchange per well with a target volume removal per cycle of 66%. To reach a final concentration of 50 mg/mL, final well volume was targeted at 1600 µL. Throughout automated buffer exchange, pressurization cycle duration was automatically adjusted by Big Tuna to have the maximum 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. Average cycle duration, initial and final well volumes, and final percent exchange were calculated in Excel.

Protein concentration

Lunatic was used to analyze the concentration of each of the four stock mAbs and each of the 24 formulations that resulted from buffer exchange on Big Tuna. Protein concentration was determined with the A280 application on Lunatic using the E1% specific to each of the four mAbs. All proteins were measured in duplicate and average concentration was reported in all cases.

Protein stability

Uncle was used to analyze the stability of each of the four stock mAbs and each of the 24 formulations that resulted from buffer exchange on Big Tuna. Protein stability was determined by dynamic light scattering (DLS) on Uncle at 20 °C.

Results

Stock protein formulations

Each of the four stock mAbs was prepared in their stock buffer at a target concentration of approximately 10 mg/mL. Table 3 shows the actual concentration and final well volume of each mAb before and after buffer exchange.

Buffer exchange

The target percent exchange of 96% for each well was reached in 10 buffer exchange cycles that averaged 27 minutes per cycle (range: 24-33 minutes/cycle). The duration of each cycle adjusted automatically so the maximum volume removed from any well per cycle was approximately the target of 66% exchange per cycle. The total time to complete the buffer exchange of four proteins each in 6 buffer formulations on a single Unfilter 24 was approximately 8.5 hours.

Following buffer exchange, 3 concentration cycles were needed to concentrate the mAbs to 50 mg/mL. The concentration cycles averaged 20 minutes per cycle (range: 10-38 minutes/cycle). The total time for 5-fold concentration on Big Tuna was approximately 1.5 hours.

Initial fill volume per well was 8000 µL. Final fill volume per well was ultrasonically measured as 1626 ± 115 µL, at the target of 1600 µL per well.

A target percent exchange of 96% was set per well, so every well must reach a minimum of 96% exchange. Because flow rate of solutions varied, some wells exchanged to >96% to ensure that all wells exchanged to at least 96%. The average percent exchange per well across the Unfilter 24 was 99.0%, with a minimum percent exchange of 96.9% and a maximum of over 99.9% (Table 4). Each of the four mAbs 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.

Protein		Before exchange	After exchange						Average after exchange
			No excipient	150 mM NaCl	150 mM sucrose	75 mM mannitol	150 mM arginine	100 mM glycine	
mAb A	Conc. (mg/mL)	10.0	51.0	42.4	54.1	56.6	48.8	56.0	51.5
	Vol. (µL)	8,000	1,593	1,928	1,621	1,586	1,765	1,573	1,678
mAb B	Conc. (mg/mL)	10.0	51.6	55.0	42.4	51.9	57.3	58.7	52.8
	Vol. (µL)	8,000	1,662	1,584	1,992	1,697	1,578	1,591	1,684
mAb C	Conc. (mg/mL)	9.9	52.1	52.9	58.8	56.8	57.6	55.1	55.6
	Vol. (µL)	8,000	1,548	1,586	1,546	1,550	1,576	1,543	1,558
mAb D	Conc. (mg/mL)	9.7	50.5	54.3	52.9	55.6	57.9	56.8	54.7
	Vol. (µL)	8,000	1,586	1,578	1,623	1,583	1,586	1,554	1,585

Table 3: Protein concentrations of each mAb formulation before and after buffer exchange as determined by the A280 application on Lunatic. Final volumes for each mAb formulation after buffer exchange as determined by the ultrasonic volume sensor on Big Tuna.

Protein	No excipient	150 mM NaCl	150 mM sucrose	75 mM mannitol	150 mM arginine	100 mM glycine
mAb A	99.5	97.5	98.9	99.5	98.0	99.6
mAb B	97.8	99.0	96.9	97.5	99.0	97.8
mAb C	>99.9	99.5	>99.9	>99.9	99.7	>99.9
mAb D	99.9	98.6	99.4	99.8	99.2	99.9

Table 4: Actual percent exchange for each sample after automated buffer exchange on Big Tuna range from 96.9% to >99.9%. Target percent exchange per pool was user-defined as 96%.

Final protein concentration

During buffer exchange on Big Tuna, the target final concentration was 5x the initial concentration of each protein, approximately 50 mg/mL.

Table 3 shows the actual concentration of each mAb before and after buffer exchange as measured by Lunatic. Actual final concentrations were approximately equal to the target of 5-fold concentration. Final concentrations paired with final well volumes show that protein recovery was high for all wells, despite any variation in concentration.

Protein stability

Antibody sizing and quality was determined by DLS measurements before and after exchange. No significant differences in size or aggregation

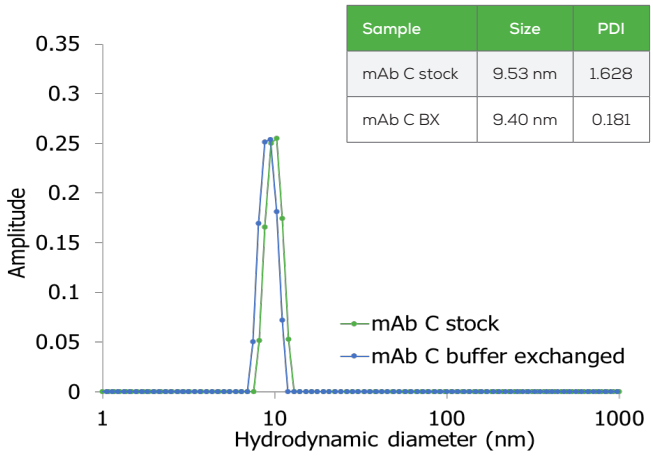

Figure 4: DLS of mAb C before and after buffer exchange on Big Tuna.

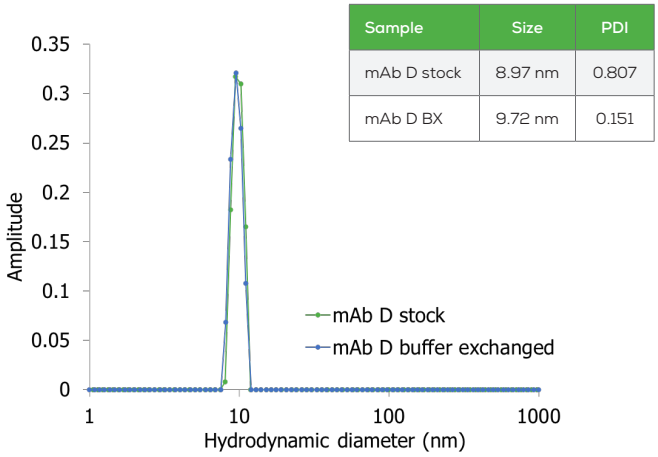

Figure 5: DLS of mAb D before and after buffer exchange on Big Tuna.

were observed. For example, DLS results of mAb C stock compared to mAb C exchanged into 10 mM histidine pH 6.0 with 100 mM glycine shows no significant differences in size or aggregation before and after exchange (Figure 4). mAb D exchanged into 10 mM histidine pH 6.0 with no excipient also showed no changes after buffer exchange (Figure 5).

Conclusion

Big Tuna is capable of conducting high-throughput platform buffer screens of multiple proteins and multiple buffer formulations with minimal hands on time. Big Tuna exchanged four mAbs into six buffer formulations and concentrated the proteins 5-fold after the exchange.

The ability of Big Tuna to adjust pressurization cycle duration after each cycle provides efficiency and prevents over-concentration 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 proteins and formulations.

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