

Meet Big Tuna: automated, versatile buffer exchange

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.

Conventional exchange methods are labor-intensive, prone to inconsistency, and difficult to manage in larger numbers. Existing methods for buffer exchange have various limitations, all of which are exacerbated at higher throughput. Automated buffer exchange systems can enable more uniform sample handling and degrees of process control that are otherwise impractical by manual methods. 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 sample 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 this process. The Unfilter 96 and Unfilter 24 are filtration plates designed to withstand 60 psi pressurization during the buffer exchange process (Figure 2). Unfilter 96 can process up to 96



Figure 1: Big Tuna automates buffer exchange.

samples ranging from 100–450 μL ; Unfilter 24 can process 24 samples ranging from 0.45–8 mL in a single run.

Buffer exchange can be an unpredictable process. The exchange rate is highly dependent on solution viscosity, which in turn is influenced by the protein, concentration, formulation, and volume. At higher throughput, samples would be expected to exchange at different rates. To maintain uniformity, the pressurization is stopped, and an ultrasonic sensor measures the volume for each sample. This measurement is used to calculate the amount of volume removed, and the amount of the new buffer to be added back before the next cycle. The actual volume removed in each well is compared to the user-defined target, and the pressurization time is adjusted, optimizing buffer exchange in real time.

Volume control on Big Tuna has the added benefit of providing a level of control that would be otherwise unavailable by manual methods. The degree of exchange per cycle, or percent buffer removal, can be used to ensure a protein is not over concentrated during the exchange, and also guards against making rapid changes to

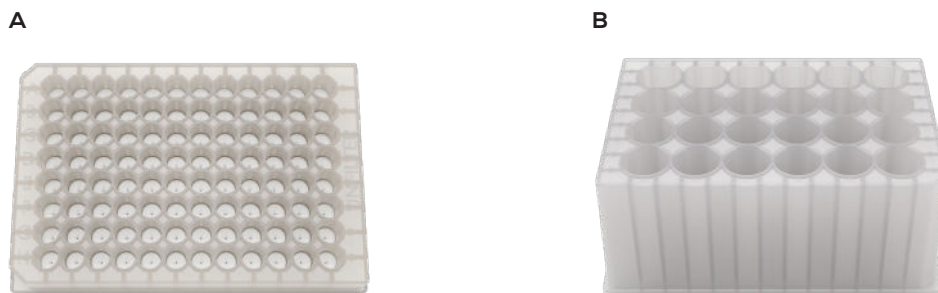


Figure 2: Big Tuna can accommodate both Unfilter 96 and Unfilter 24. **A:** Unfilter 96 allows for up to 96 samples to be buffer exchanged simultaneously at volumes of 100-450 μ L per well. **B:** Unfilter 24 allows for up to 24 samples to be buffer exchanged simultaneously at volumes of 0.45-8 mL per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

the formulation that would cause other aggregation events. The user can control the level of the exchange based on their application needs. With low protein concentrations, or exchange into similar buffers, the % removal per cycle can be set high to finish the exchange faster. With high concentrations of protein, proteins sensitive to aggregation, or large formulation changes, a lower % removal can be used. This would increase the exchange time, but also ensure a quality protein at the end of the process.

Big Tuna automates and minimizes hands-on time. Before the run, the Unfilter 96 or Unfilter 24 is filled with protein to be exchanged and placed in the exchange chamber, and new buffers are placed on the deck. During the run, Big Tuna alternates between filtration, volume measurement, and new buffer addition (Figure 3).

In this application note, proof-of-concept experiments were run on Big Tuna, demonstrating the ability to perform buffer exchange in multiple

formats and nail final concentration targets that would be impossible to do with manual methods.

Methods

Protein and buffer preparation

Human IgG (hlgG) was nominally prepared at 10 mg/mL or 100 mg/mL in PBS, pH 6.8. Proteins were manually pipetted into a 10 kDa Unfilter 96 or Unfilter 24 before the start of each buffer exchange. Proteins were buffer exchanged into PBS, pH 6.8.

Protein concentration

Lunatic was used to analyze the concentration of all samples before and after buffer exchange. Protein concentration was determined with the A280 application on Lunatic using the E1% specific to hlgG. Final concentrations were measured for each well and are reported as the average concentration across the Unfilter 96 or Unfilter 24 \pm standard deviation.

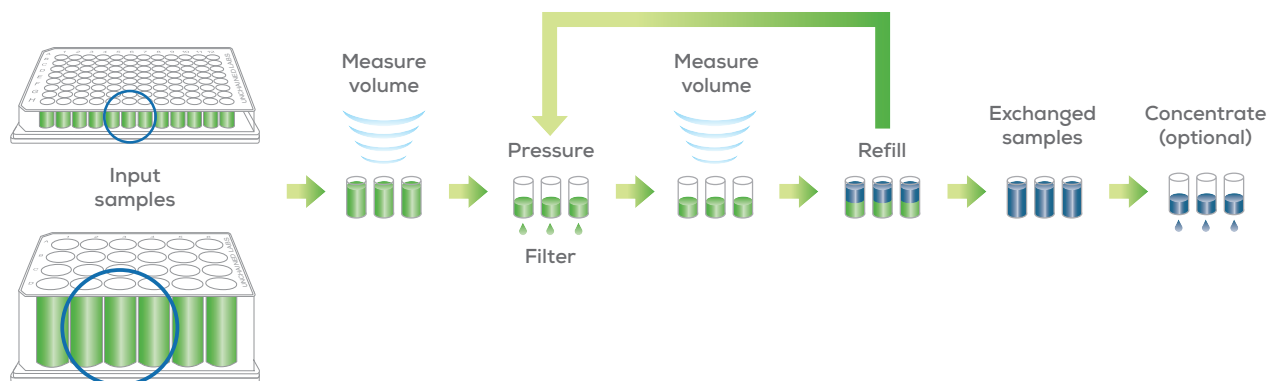


Figure 3: Big Tuna uses a pressure-based UF/DF method with gentle orbital mixing to buffer exchange proteins with the Unfilter 96 or Unfilter 24.

Buffer exchange capabilities

Big Tuna enables the operator to set a total % exchange up to 99%. For all experiments in this application note, the exchange % was set to 96% total exchange. When sample wells were identified as duplicates, the total exchange for the pool was set to 96%.

Big Tuna allows the operator to choose the % volume removal per cycle. All experiments used 66% removal per cycle, except for experiments comparing Dilute and Filter (50% removal/cycle) and Filter and Refill (25% removal/cycle).

Other capabilities for buffer exchange methods are described within their respective experiments. Big Tuna software was used for experimental design and execution. Results were logged, and further concentration analysis was performed offline.

Results

Buffer exchange with Unfilter 96

A stock of hlgG (10.5 mg/mL) was buffer exchanged into PBS, pH 6.8. 450 μ L was manually transferred to each well of an Unfilter 96. The target percent exchange of 96% for each pool was reached in 3 cycles of approximately 20 minutes each. The average percent exchange across the Unfilter 96 was 96.3% (Table 1). The total run time to complete the buffer exchange of one protein exchanged into one buffer on an Unfilter 96 was 2.6 hours.

Final fill volume per well was similar to the initial fill volume per well at 457 ± 9 μ L, showing consistency across the Unfilter 96 and no significant difference between the initial and final fill volume per well. Following buffer exchange the average concentration across the Unfilter 96 was 10.4 ± 0.2 mg/mL, right on target (Table 1).

Variable	Initial	Target final	Actual final
Conc. (mg/mL)	10.5	10.5	10.4 ± 0.2
Well fill vol. (μ L)	450	450	457 ± 9
% exchange	–	>96	96.3

Table 1: Big Tuna buffer exchanges human IgG into PBS in 96 wells of an Unfilter 96 at 450 μ L per well.

Buffer exchange and 3x concentration with Unfilter 96

For this experiment protein was exchanged into buffer and then concentrated 3-fold after exchange was completed. A stock of hlgG (9.7 mg/mL) was buffer exchanged into PBS pH, 6.8. 450 μ L was manually transferred to each well of an Unfilter 96. The target percent exchange of 96% for each pool was reached in 4 cycles of approximately 18 minutes each. The duration of each cycle was adjusted automatically so the average volume removed per cycle was approximately the target of 66% exchange per cycle. The average percent exchange across the Unfilter 96 was 98.6% (Table 2).

Human IgG was concentrated 3-fold after buffer exchange, with a target final concentration of 29.1 mg/mL. Following buffer exchange, 1 concentration cycle was needed to concentrate the protein to the target of 29.1 mg/mL. The concentration cycle was about 1 minute long.

The final average concentration across the Unfilter 96 was 31.9 ± 2.5 mg/mL, slightly above target. Final fill volume per well was targeted to 150 μ L, due to the 3-fold concentration step. The final fill volume per well was similar to the target at 146 ± 5 μ L (Table 2).

Variable	Initial	Target final	Actual final
Conc. (mg/mL)	9.7	29.1	31.9 ± 2.5
Well fill vol. (µL)	450	150	146 ± 5
% exchange	–	>96	98.6

Table 2: Big Tuna buffer exchanges human IgG into PBS in 96 wells of an Unfilter 96 at 450 µL per well, followed by 3-fold concentration to 150 µL.

The total run time to complete the buffer exchange of one protein exchanged into one buffer followed by a 3-fold concentration step on an Unfilter 96 was 3.5 hours.

Buffer exchange with Unfilter 24

A stock of hIgG (10.8 mg/mL) was buffer exchanged into PBS, pH 6.8. 8 mL was manually transferred to each well of an Unfilter 24. The target percent exchange of 96% for each pool was reached in 3 cycles of approximately 58 minutes (56–60 minute range). The average percent exchange across the Unfilter 24 was 97.7% (Table 3). The total run time to complete the buffer exchange of one protein exchanged into one buffer on an Unfilter 24 was 4.3 hours.

Final fill volume per well was similar to the initial fill volume per well at 8,001 ± 73 µL, consistent across the plate with no significant difference between the initial and final fill volume. Following buffer exchange the average concentration across the Unfilter 24 was 10.9 ± 0.1 mg/mL, right on target (Table 3).

Variable	Initial	Target final	Actual final
Conc. (mg/mL)	10.8	10.8	10.9 ± 0.1
Well fill vol. (µL)	8,000	8,000	8,001 ± 73
% exchange	–	>96	97.7

Table 3: Big Tuna buffer exchanges human IgG into PBS in 24 wells of an Unfilter 24 at 8,000 µL per well.

Buffer exchange and 3x concentration with Unfilter 24

For this experiment protein was exchanged into buffer and then concentrated 3-fold after exchange was completed. A stock of hIgG (9.8 mg/mL) was buffer exchanged into PBS, pH 6.8. 8 mL was manually transferred to each well of an Unfilter 24. The target percent exchange of 96% for each pool was reached in 3 cycles of approximately 60 minutes (58–62 minute range). The duration of each cycle was adjusted automatically so the average volume removed per cycle was approximately the target of 66% exchange per cycle. The average percent exchange across the Unfilter 24 was 97.0% (Table 4).

Human IgG was concentrated 3-fold after buffer exchange, with a target final concentration of 29.4 mg/mL. Following buffer exchange the average concentration across the Unfilter 24 was 32.2 ± 0.5 mg/mL, slightly above target. Final fill volume per well as measured by the on-deck volume sensor was targeted to 2,667 µL, due to the 3-fold concentration step. The final fill volume per well was similar to the target at 2,680 ± 15 µL (Table 4).

Variable	Initial	Target final	Actual final
Conc. (mg/mL)	9.8	29.4	32.2 ± 0.5
Well fill vol. (µL)	8,000	2,667	2,680 ± 15
% exchange	–	>96	97.0

Table 4: Big Tuna buffer exchanges human IgG into PBS in 24 wells of an Unfilter 24 at 8,000 µL per well, followed by 3-fold concentration to 2,667 µL.

The total run time to complete the buffer exchange of one protein exchanged into one buffer followed by a 3-fold concentration step on an Unfilter 24 was 4.4 hours.

Filter and Refill buffer exchange method

Filter and Refill is the default method, which will first concentrate the protein to the % volume removal target, then add new buffer back to the original volume.

A stock of hlgG (99.6 mg/mL) was buffer exchanged into PBS, pH 6.8. 4 mL was manually transferred to each well of an Unfilter 24. The target percent exchange of 96% for each pool was reached in 12 cycles of approximately 110 minutes per cycle (range: 99.4 - 126.6 minutes/cycle). The duration of each cycle was adjusted automatically so the average volume removed per cycle was approximately the target of 25% exchange per cycle. The average percent exchange across the Unfilter 24 was 96.9% (Table 5).

The final fill volume per well was similar to the target at 3,993 ± 25 µL. Following buffer exchange the average measured concentration across the Unfilter 24 was 100.9 ± 1.6 mg/mL, right on target (Table 5).

Variable	Initial	Target final	Actual final
Conc. (mg/mL)	99.6	99.6	100.9 ± 1.6
Well fill vol. (µL)	4,000	4,000	3,993 ± 25
% exchange	–	>96	96.9

Table 5: Big Tuna buffer exchanges human IgG into PBS in 24 wells of an Unfilter 24 at 4,000 µL per well, using the Filter and Refill method. Filter and Refill first concentrates protein down to target, then refills wells with the new buffer in each cycle.

The total run time to complete the buffer exchange of a high concentration protein using the Filter and Refill method in an Unfilter 24 was 24.6 hours.

Dilute and Filter buffer exchange method

Dilute and Filter will dilute the starting protein with the new buffer, then start the exchange. Dilute and Filter may enable higher concentration exchanges to process faster.

A stock of hlgG (99.7 mg/mL) was buffer exchanged into PBS, pH 6.8. 4 mL was manually transferred to each well of an Unfilter 24. Big Tuna diluted each well with 4 mL of PBS, to a total of 8 mL. The target percent exchange of 96% for each pool was reached in 5 cycles of approximately 115 minutes per cycle (range: 1.3 - 145.2 minutes/cycle). The duration of each cycle was adjusted automatically so the average volume removed per cycle was approximately the target of 50% exchange per cycle. The average percent exchange across the Unfilter 24 was 97.0% (Table 6).

The final fill volume per well was similar to the target at 4,009 ± 18 µL. Following buffer exchange the average concentration measured across the Unfilter 24 was 98.7 ± 1.3 mg/mL, right on target (Table 6).

Variable	Initial	Target final	Actual final
Conc. (mg/mL)	99.7	99.7	98.7 ± 1.3
Well fill vol. (µL)	4,000	4,000	4,009 ± 18
% exchange	-	>96	97.0

Table 6: Big Tuna buffer exchanges human IgG into PBS in 24 wells of an Unfilter 24 at 4,000 µL per well, using the Dilute and Filter method. Dilute and Filter first dilutes each well with new buffer then concentrates protein down to target in each cycle.

The total run time to complete the buffer exchange of a high concentration protein using the Dilute and Filter method in an Unfilter 24 was 11.4 hours, less than half the time of the Filter and Refill method. By diluting the protein before filtering it, viscosity is decreased, improving the flow rate. With a diluted protein sample, a higher percent volume removal per cycle is possible, further decreasing the total buffer exchange time. If a larger volume of protein needs to be exchanged, the default Filter and Refill method will exchange protein effectively over a longer period of time (Table 7).

Variable	Filter & refill method	Dilute & filter method
Total run time (hours)	24.6	11.4
Time under pressurization (hours)	22.1	9.5
Number of cycles	12	5

Table 7: High protein concentration buffer exchange using Filter and Refill versus using Dilute and Filter on Big Tuna. At high protein concentrations, viscosity slows buffer exchange. The Dilute and Filter method dilutes proteins before buffer exchange, decreasing viscosity and total buffer exchange time.

Conclusion

Big Tuna allows for automated buffer exchange of up to 96 samples of 100–450 µL, or up to 24 samples of 0.45–8 mL in a single experiment. It is the only automated buffer exchange platform to accommodate both low- and high-volume samples.

Big Tuna is capable of conducting high-throughput buffer exchange in multiple formats with minimal hands on time. It allows for flexible buffer exchange procedures by letting users select their desired percent exchange, volume removed per cycle, buffer exchange method, and final concentration. Initial and final protein conditions, such as concentration, well volume, and percent exchange showed consistency across 10 kDa Unfilter 96 and Unfilter 24 in all six experiments conducted here.



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

© 2019 Unchained Labs. All rights reserved. Big Tuna is a trademark and Unchained Labs is a registered trademark of Unchained Labs. All other brands or product names mentioned are trademarks owned by their respective organizations.