Meet Big Tuna: automated, multiplexed, versatile plate-based buffer exchange

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Introduction

Buffer exchange is an essential step for all biologics preparatory work. Conventional exchange methods are labor intensive, prone to recovery challenges, and difficult to manage in larger numbers. We developed Big Tuna, an automated, flexible buffer exchange system, to address these challenges.

Big Tuna uses a combination of pressure-based ultrafiltration/diafiltration (UF/DF) and orbital mixing (Figure 1). An integrated ultrasonic sensor measures volumes during the exchange process without making physical contact with samples.

Big Tuna can buffer exchange up to 96 samples from 100-450 µL each or up to 24 samples from 0.45-8 mL each using Unfilter (Figure 2). Here we demonstrate how Big Tuna can exchange multiple proteins into multiple buffers with uniform results and high protein recovery.

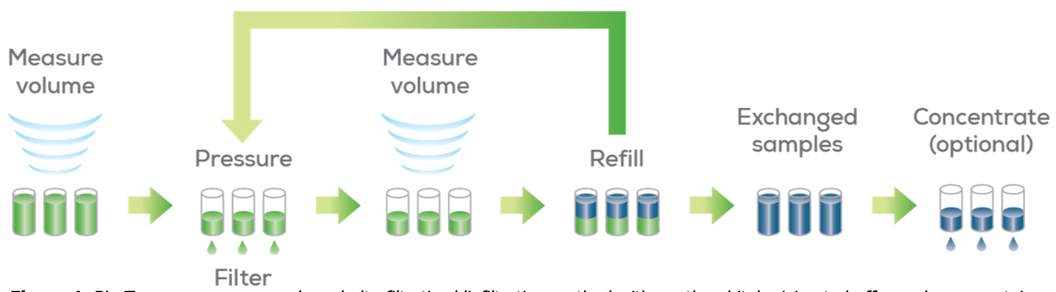

Figure 1. *Big Tuna uses a pressure-based ultrafiltration/diafiltration method with gentle orbital mixing to buffer exchange proteins.*

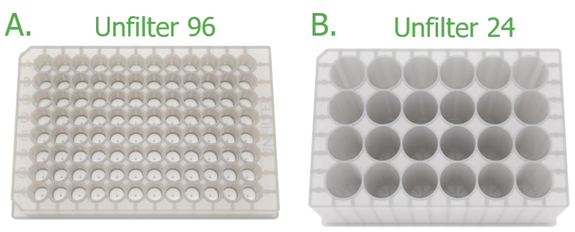

Figure 2. *96- and 24-well formats enable exchanges between 100 µL and 8 mL per sample.*

Materials and Methods

Protein and buffer preparation

- Four stock monoclonal antibodies (mAbs), nominally at 10 mg/mL, were prepared in their stock buffers and transferred to an Unfilter 24
- 8 mL of each mAb was automatically exchanged into 6 formulations (Table 1)

Buffer exchange and final concentration step

- Key buffer exchange parameters were user defined (Table 2)
- Pressurization cycle duration automatically adjusted throughout the run
- Protein was concentrated to 50 mg/mL after buffer exchange
- Initial and final protein concentrations were measured in duplicate on Lunatic

Protein stability

- DLS measured at 20 °C on Uncle to confirm stability before and after exchange

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. *4 antibodies were simultaneously exchanged against 6 buffers, for 24 total tested conditions.*

Parameter	Setting
Target exchange percentage	96%
Target volume removed per cycle	67%
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 for this experiment. Pressurization cycle duration automatically adjusted to reach a maximum of 67% volume removed from any well.*

Results

Buffer exchange

- After 10 cycles actual percent exchange ranged from 96.9% to >99.9%
- Average cycle duration of 27 minutes, range of 24-33 minutes
- Total run time for buffer exchange was approximately 8.5 hours
- Initial well volume was 7958 ± 60 µL (target: 8000 µL)
- Final well volume was 1623 ± 126 µL (target: 1600 µL)

Concentration to target

- Total time for protein concentration was about 1.5 hours (100 min)
- Average concentration cycle duration of 20 minutes, range of 10-38 minutes
- Actual final protein concentration was highly accurate (Table 3)

Protein	Initial conc. (mg/mL)	Final conc. (mg/mL)						Average final conc. (mg/mL)
		No excipient	150 mM NaCl	150 mM sucrose	75 mM mannitol	150 mM arginine	100 mM glycine	
mAb A	10.0	51.0	42.4	54.1	56.6	48.8	56.0	51.5
mAb B	10.0	51.6	55.0	42.4	51.9	57.3	58.7	52.8
mAb C	9.9	52.1	52.9	58.8	56.8	57.6	55.1	55.6
mAb D	9.7	50.5	54.3	52.9	55.6	57.9	56.8	54.7

Table 3. *Protein concentrations of each mAb formulation before and after buffer exchange.*

Protein stability

- Automated buffer exchange did not affect protein quality
- Protein quality was assessed by DLS on Uncle after buffer exchange on Big Tuna
- No significant differences in sizing or aggregation before and after buffer exchange (Figures 3 and 4)

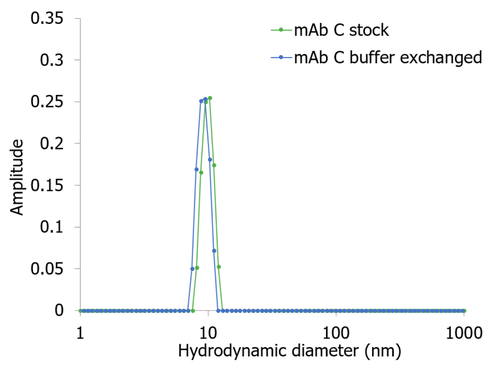

Figure 3. *DLS of mAb C before and after buffer exchange on Big Tuna.*

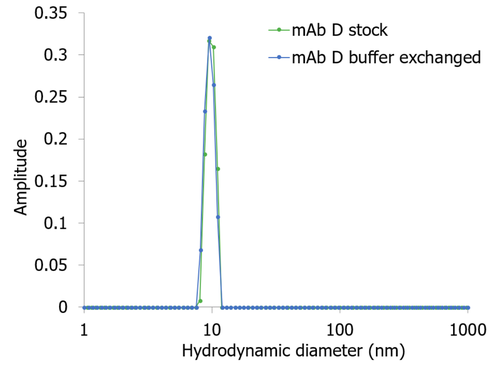

Figure 4. *DLS of mAb D before and after buffer exchange on Big Tuna.*

Conclusions

- Big Tuna can perform high-throughput buffer exchange with multiple proteins and buffers
- Exchange rates are viscosity-dependent, which is impacted by formulation, protein, and concentration
- Big Tuna optimizes the pressurization cycle duration in real time so all samples reach the exchange target while preventing any samples from drying out or over-concentrating
- With the UF/DF method, the operator can control % volume removed per cycle
- Initial and final protein conditions are consistent across the Unfilter 24 despite protein and formulation differences

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