Meet Lil' Tuna: Automated plate-based benchtop buffer exchange

Introduction

Protein researchers rely on buffer exchange (BX) and concentration steps at every stage of the process of discovering and developing their candidates. From purification to functional assays to formulation screening, BX is always part of the workflow. Traditional methods introduce variability, require a lot of handling and setup, or run only one sample at a time. That can turn routine sample prep into a major bottleneck, especially when scientists need to process many candidates and conditions.

Lil' Tuna automates plate-based BX and concentration on the benchtop, giving scientists hands-off process control of 1–96 samples with better than 96% protein recovery (Figure 1). High-throughput, parallel processing allows for running more samples in less time, making it ideal for preformulation or formulation screening, stability testing and developability studies, or reducing variability in sample prep before analytical assays. The small form factor and easy-to-use software simplify stand-alone usage while its API and easily accessible plate-based consumable help it easily integrate with other automation solutions.

Lil' Tuna uses pressure-based ultrafiltration/diafiltration (UF/DF) to make the BX process faster, less manual, and more consistent (Figure 2).[1–3] Each run starts by measuring the volume in each well with an ultrasonic sensor. Lil' Tuna then lowers the samples into a chamber and pressurizes it with air while shaking them to send the filtrate through the membrane on the bottom of the well, keeping the flow fast and uniform while preventing build-up at the membrane interface, also called membrane fouling. This combination of pressure and mixing reduces the risk of process-related aggregation. Then, the volume is checked again, new buffer gets added, and the cycle time automatically adjusts based on how much liquid was removed. Adjusting the cycle duration gives real-time process optimization and control, since

Figure 1: Lil' Tuna is the plate-based benchtop buffer exchange system for either automation-ready or stand-alone workflows.

the amount of retentate is known at every step. This cycling repeats until samples reach the target exchange percent and volume, with an optional concentration step at the end. Running the system is simple: set the target exchange, final volume, and sample format—then walk away. Lil' Tuna handles the rest.

The same automated UF/DF process runs across consumables so you can scale Lil' Tuna to adapt to your needs. For large screens, Unfilter 96 handles 96 samples in an SBS-format plate with volumes ranging from 100–450 µL per well (Figure 3A). Unfilter 24 holds up to 24 samples ranging from 0.45–8 mL in an SBS-format plate (Figure 3B). For smaller scale, run 1–6 Unas at a time, each holding between 0.45 and 8 mL of sample (Figure 3C).

Una and Unfilter membranes are available with 10, 30, and 100 kDa molecular weight cutoffs (MWCO), while the Unfilter 96 is also available in a 3 kDa option. Membranes are made from regenerated cellulose (RC), which minimizes protein interactions and keeps exchange yields high. Unas are also available with 30 and 100 kDa MWCO polyethersulfone (PES) membranes, in case your sample is more compatible with PES. This range of

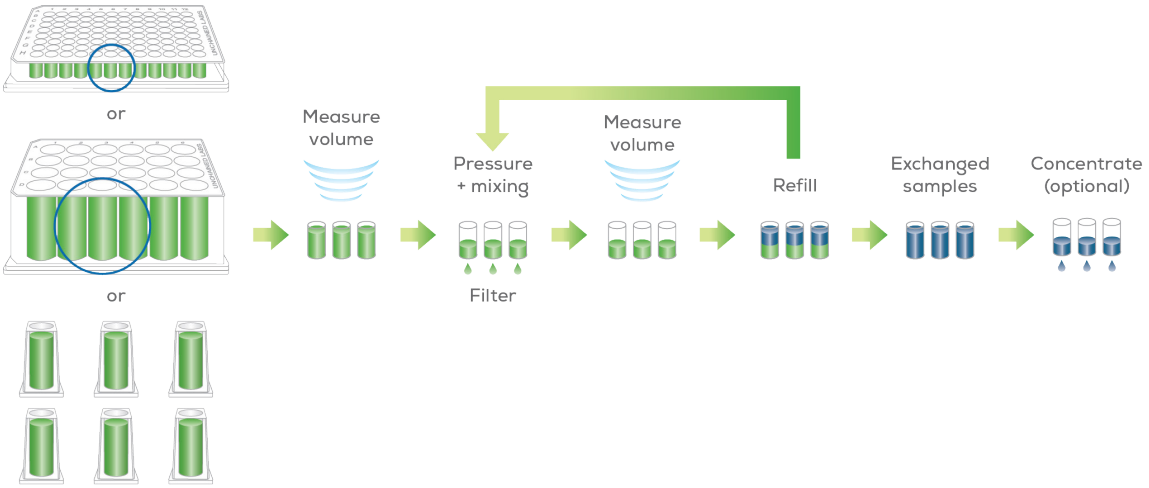


Figure 2: Lil' Tuna uses the tried-and-tested pressure-based approach to UF/DF. Each cycle starts with a sample volume check using an ultrasonic sensor. Next, the samples are pressurized with gentle mixing to remove filtrate which prevents dead-end filtration and membrane fouling. The volume is then checked again and Lil' Tuna refills each well with your exchange buffer. This cycle repeats until your samples reach the target exchange percent and volume.

consumable options allows you to find the right fit for your sample volume, throughput, size, and chemistry requirements while achieving ≥96% sample recovery.

In this application note, you'll see that Lil' Tuna buffer exchanges proteins and antibodies to precise percent exchanged, concentration, and volume targets with high recovery in Unfilter 24 and 96 while preserving sample quality.

Methods

Human IgG (hIgG) was reconstituted with aqueous 0.9% NaCl to nominal concentrations of 0.1, 1, and 10 mg/mL. Bovine serum albumin (BSA) was reconstituted with phosphate-buffered saline (PBS) to 10 mg/mL. The proteins and volumes indicated in each experiment were added to either pre-wetted regenerated cellulose 30 kDa MWCO Unfilter 24, 10 kDa MWCO Unfilter 96, or 30 kDa MWCO Unfilter 96 and buffer exchanged into PBS.

For the buffer exchange experimental setup the default parameters for buffer exchange of proteins with concentrations 0.5–50 mg/mL were used: removal of 66% of the volume per cycle with 700 rpm shaking and a target exchange rate of 96%. Initial and final protein concentration and size were checked on Stunner with buffer blanks using an E1% of 13.7 for IgG and 6.67 for BSA. Total runtime, initial, and final volumes were measured and % exchange was calculated by Lil' Tuna.

Figure 3: Lil' Tuna consumables are designed to suit your needs. Unfilter 96 (**A**) handles as many as 96 samples of 100–450 µL. Unfilter 24 (**B**) processes up to 24 samples between 0.45 and 8 mL of sample, while Unas (**C**) keep things light with runs of 1-6 samples at a time in the same volume range as Unfilter 24.

	Initial	Target	Final
Fill Volume (µL)	428 ± 9.7	450	441 ± 15
% exchange	–	96	99.7 ± 0.3
% recovery	–	≥96	100 ± 2
Z-average diameter (nm)	7.1	–	7.2 ± .3

Table 1: Initial, target, and final fill volume, % exchange, % recovery, and Z-average diameter of BSA exchanged from PBS into PBS using Unfilter 96, 10 kDa MWCO. Final values and initial fill volume are the average of 96 wells ± 1 standard deviation.

All particle size results were collected by dynamic light scattering (DLS) using Stunner. The system default buffer viscosities of 0.92 and 0.905 cP were used for PBS and 0.9% NaCl, respectively, and the same refractive index of 1.334 at 25 °C was used for both. The Z-average diameter at a scattering angle of 144° was collected and reported.

Percent recovery was calculated from the initial and final volume measured on Lil' Tuna, and the initial and final concentration measured on Stunner using the following equation:

$$\%\ recovery = \frac{final\ volume \times final\ concentration}{initial\ volume \times initial\ concentration} \times 100$$

Results

Manually removing elution buffers or setting up formulation stability studies from modern high-throughput protein production workflows is a major productivity bottleneck. Lil' Tuna solves that problem with its Unfilter 96 to exchange and concentrate up to 96 samples at a time in volumes of 100–450 µL. A stock of 10 mg/mL BSA was buffer exchanged into PBS in all the wells of an Unfilter 96. The final fill volume and exchange rate were spot on, within 4% of the target values, and the % recovery exceeded the target (Table 1); 95 of 96 wells had ≥96% recovery.

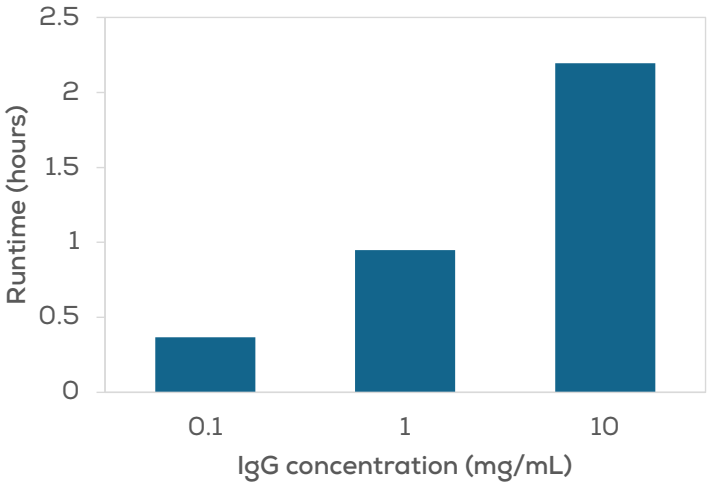
The z-average diameters of the BSA samples were similar before and after exchange.

After an initial round of candidate or formulation screening, the numbers of protein samples usually shrinks, but their volumes tend to increase. Unfilter 24 plates let Lil' Tuna transition from low volume, high-throughput to medium volume sample processing just as you do. After buffer exchange of 10 mg/mL IgG from 0.9% NaCl to PBS with an Unfilter 24, the final volumes and % exchange were on-target (Table 2). Z-average diameters were consistent before and after exchange and the protein recovery was higher than 96% for all wells. With the membrane MWCO to match your sample, Lil' Tuna excels in processing proteins and antibodies, big or small.

Setting up exchanges on Lil' Tuna takes about 15 minutes of hands-on time. During the run, the ultrasonic sensor tracks volume changes in each well and adapts the cycle duration in real time to speed up the process and keep everything on-target. Flow rates vary with factors including initial protein concentration, so this level of system control helps Lil' Tuna handle samples from early discovery to formulation at a wide range of concentrations. Buffer exchanges of 8 wells of 0.1, 1, and 10 mg/mL hIgG in a 30 kDa MWCO Unfilter 96 took less than 2.2 hours and all tested concentrations remained close to the starting concentration, exhibited on-target %

	Initial	Target	Final
Fill Volume (µL)	7.7 ± 0.03	8	7.2 ± 0.08
% exchange	–	96	96.6 ± 0.3
% recovery	–	≥96	100 ± 1
Z-average diameter (nm)	12.6	–	13.3 ± 0.4

Table 2: Initial, target, and final fill volume, % exchange, % recovery, and Z-average diameter of human IgG from 0.9% NaCl into PBS using Unfilter 24, 30 kDa MWCO. Initial fill volume and final values are the average of 24 wells ± 1 standard deviation.



	0.1 mg/mL	1 mg/mL	10 mg/mL
Final concentration (mg/mL)	0.1 ± 0.002	1.0 ± 0.03	10.5 ± 0.2
% exchange	96.7 ± 0.3	98.7 ± 0.5	98.5 ± 1.2
% recovery	97 ± 2	98.3 ± 2.6	96.2 ± 2.2

Figure 4: Total runtime, final concentration, % exchange, and % recovery of 8 wells each of 0.1, 1, and 10 mg/mL hIgG in 0.9% NaCL to PBS in Unfilter 96, 30 kDa MWCO. Values are the average of 8 wells ± 1 standard deviation.

exchange, and high protein mass recoveries of ≥96% (Figure 4). No matter the concentration, the process is hands-off after setup, freeing scientists to focus on other, higher-value, work.

Conclusion

Lil' Tuna automates buffer exchange and concentration so scientists can spend more time generating decision-ready data. Flexible consumable formats support 1–96 samples at a time, giving teams a benchtop workflow that accommodates everything from large, plate-based screens or medium-volume sample processing to small, precious sample sets. Just set the target percent removal and exchange and let Lil' Tuna's built-in process control keep everything steady. Integrate BX with other lab automation through Lil' Tuna's API and SBS-format plates that are readily accessible to liquid- and plate-handling robot systems. Single-use wells help reduce cross-contamination risk, while multiple MWCOs and membrane materials make it easy to match the consumable to the molecule, volume, and application. With gentle pressure-based UF/DF, real-time volume tracking, and adaptive cycle durations, Lil' Tuna delivers consistent exchange, retains sample quality, and yields ≥96% recovery across protein and mAb workflows.

References

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