INTip SEC—Guanidine Buffer Exchange

SEC

HIGHLIGHTS: High Reproducibility, High Throughput, Seamless Integration

PURPOSE AND OBJECTIVE

- Demonstrate the utility of INTip SEC for 8M Guanidine buffer exchanged for PBS.
- Recover greater than 90% of the protein from 150 µL sample.
- Automated protocol including INTip Swelling to avoid messy and time-consuming centrifugation steps.
- Process up to 96 samples in under 30 minutes.

METHOD

Guanidine is a common buffer constituent used to denature protein for downstream analysis. However, buffer exchange is often required because guanidine and other chelating buffers are detrimental to protein analysis. Buffer exchange can be accomplished in a variety of ways, but the most common is group separation by size exclusion chromatography. Traditional size exclusion technologies are manual, laborious and time consuming. Tip-based size exclusion technology allows for an automated method for buffer exchange. DPX SEC tips are a unique and improved tip-based SEC product because they utilize INTip Swelling. INTip swelling is a patent-pending automated swelling of the SEC sorbent. The SEC tips are manufactured and shipped dry to avoid messy packaging and centrifugation steps.

In this specific application, 8M guanidine was chosen as the sample composition to showcase the capacity of the DPX SEC tips for removing these common buffers. 150 µL of 8M guanidine with a range of covalently labeled proteins (10-250 kDa) was applied to the swollen SEC tip. An additional 100 µL of phosphate buffered saline (PBS) was added to load the sample into the column. Finally, the protein was eluted into 200 µL of PBS, providing a minimally diluted workable solution for downstream applications. For this study, two serial applications of 50 µL of PBS were added to the column after the 200 µL PBS elution to monitor guanidine and protein separation.

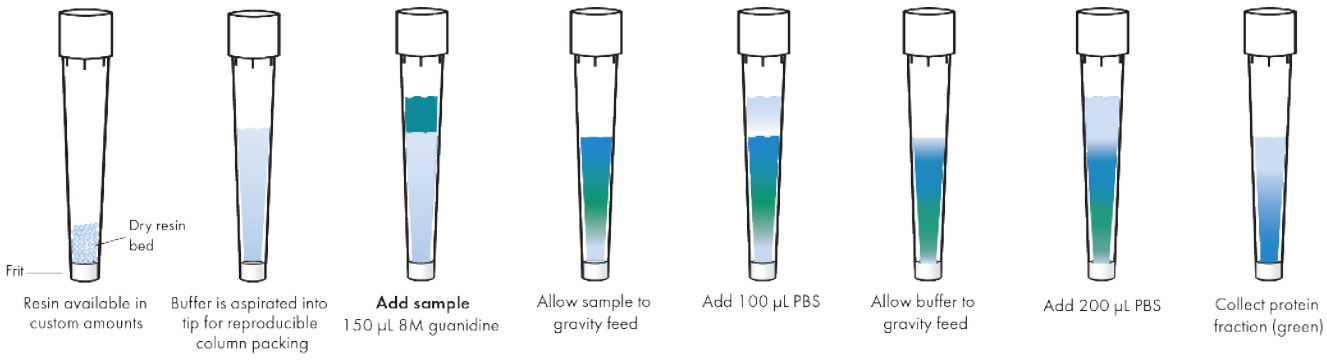
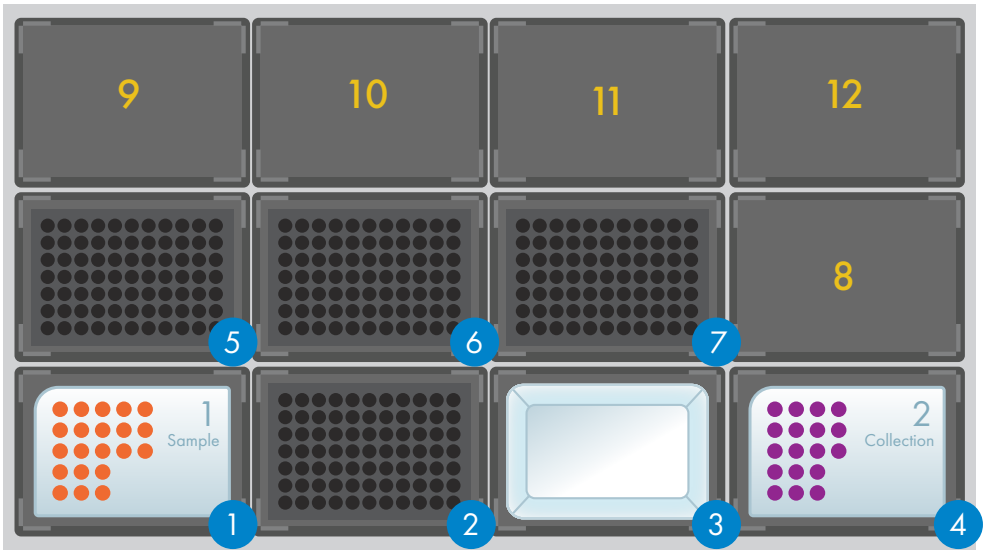


Figure 1. Schematic represents the automated INTip SEC protocol for 150 µL 8M guanidine buffer exchange



1. Sample
2. Buffer- INTip Swelling
3. PBS Buffer
4. Final Collection Plate

Location 2 and 4 are Tip Isolator Pedestals from Hamilton (P/N 65422-01) which allows for the SEC tips to be loaded on top of a buffer reservoir or collection plate, respectively.

5. 300 µL CO-RE Tips
6. Funnel Plate
7. 300 µL CO-RE Tips

Figure 2. Hamilton Microlab Nimbus96 deck layout for processing 96 samples for buffer exchange

RESULTS AND DISCUSSION

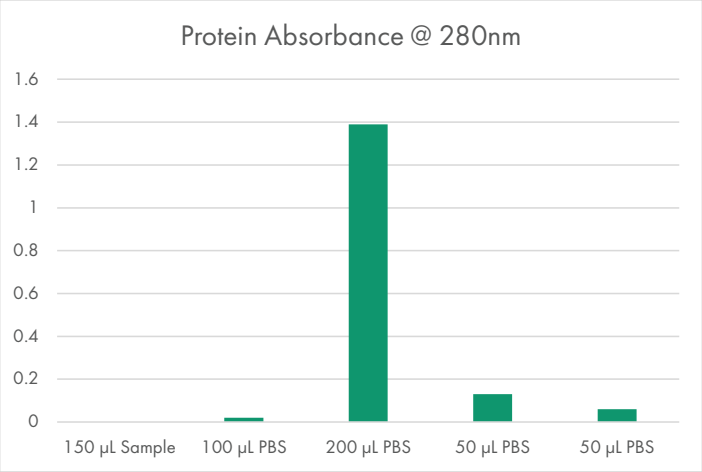


Figure 3. Evaluation of protein content in the flow through after sample addition, 100 µL PBS addition, 200 µL PBS addition (elution), and two subsequent 50 µL PBS additions.

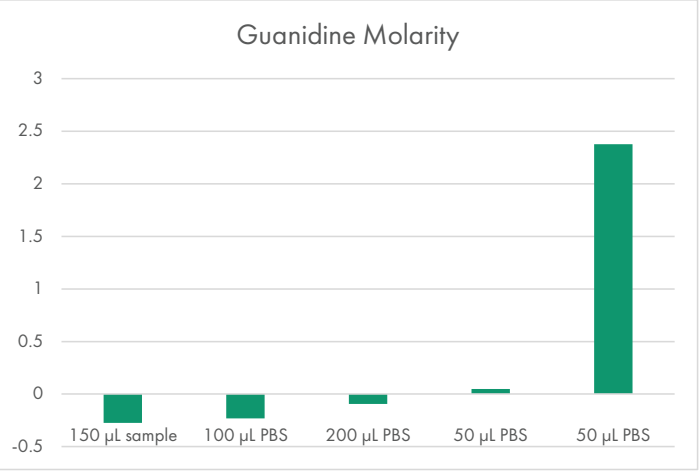


Figure 4. Evaluation of guanidine in the flow through after sample addition, 100 µL PBS addition, 200 µL PBS addition (elution), and two subsequent 50 µL PBS additions.

The target covalently labeled proteins were shown to elute with high recovery (approximately 79%) in the 200 µL PBS fraction as shown in Figure 3. Further, guanidine was retained on the SEC tip column and shown not to elute during the 200 µL collected fraction as shown in Figure 4. Guanidine did not begin to elute from the column until the second elution of 50 µL PBS. Therefore, the elution volume could be increased to 250 µL and still be free of Guanidine.

This buffer exchange application shows that the DPX SEC tips can be utilized for common buffer exchange scenarios (group separation) up to a concentration of 8M. The SEC tips provide a workable protein fraction with minimal dilution factor.

DPX SEC tips are offered in 1500 Da and 5000 Da cutoff options with sample volume compatibilities ranging 50 µL to 250 µL.