

# Automated Desalting and Buffer Exchange of Human IgG Protein in 6M Guanidine

**HIGHLIGHTS:** Process 96 samples in < 15 minutes



## PURPOSE AND OBJECTIVE

- Demonstrate the utility of INTip™ Size Exclusion Chromatography (SEC) for the purification of human IgG protein from 6 M guanidine.
- Recover greater than 85% of IgG with undetectable guanidine in final elution with a 1:1 ratio of sample to elution volume.
- Show consistent column swelling resulting in low % RSD values.
- Demonstrate the benefits of patent-pending INTip Swelling and how the technology simplifies SEC workflows.

## METHOD

DPX's SEC Tip technology incorporates traditional size exclusion chemistry in a pipette tip for automated and efficient group separation, desalting, buffer exchange, and protein purification. High salt content is necessary in many workflows for the denaturation of proteins prior to downstream analysis. Unfortunately, salt can cause detrimental effects if present during final analysis steps. Size exclusion chromatography is utilized for the purification of proteins from their high salt content environments and exchanging them into a required buffer for analysis. DPX SEC tips are optimized to provide efficient recovery of target proteins above the media cutoff (1500 Da or 5000 Da).

Immunoglobulins are glycoproteins called antibodies which are produced when plasma cells work to mediate immunity by binding to specific antigens. This characteristic makes monoclonal antibodies (mAb) a commonly used drug delivery system for clinical therapeutics. Among the five isotypes of immunoglobulin antibodies found in human serum, Immunoglobulin G (IgG) is most common, representing 75% of all immunoglobulins in humans. IgG is also a primary focus for drug discovery and development due to its long half-life. Around 160,000 Da in molecular weight, IgG antibody has been chosen to validate the use of SEC Tips for these common applications in the pharmaceutical industry. This automated method was performed with a Hamilton STAR automated liquid handler (ALH). SEC Tips were first pierced on the deck with a piercing tool shown in **Figure 1**. An example of a pierced barrier is shown in **Figure 2**.

Automated piercing and the DPX patent-pending process for INTip Swelling eliminates all pre-ALH steps such as slurry preparation, column packing, centrifugation, and manual cap or plug removal.

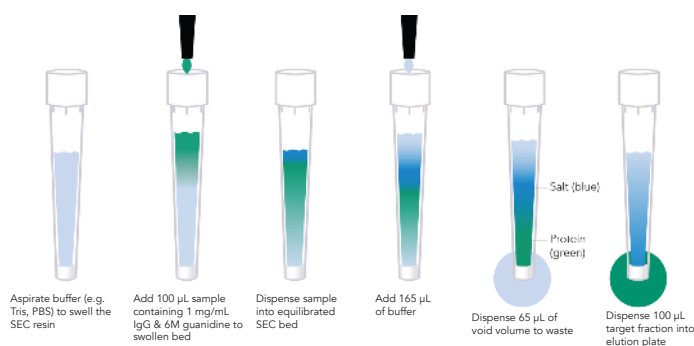


**Figure 1.** Piercing tool from DPX Technologies designed for compatibility with Hamilton Robotics platforms. Allows for on-deck piercing which replaces manual cap or plug removal.



**Figure 2.** Image comparing the intact pierceable barrier and a barrier pierced barrier with after using the tool shown in **Figure 1**.

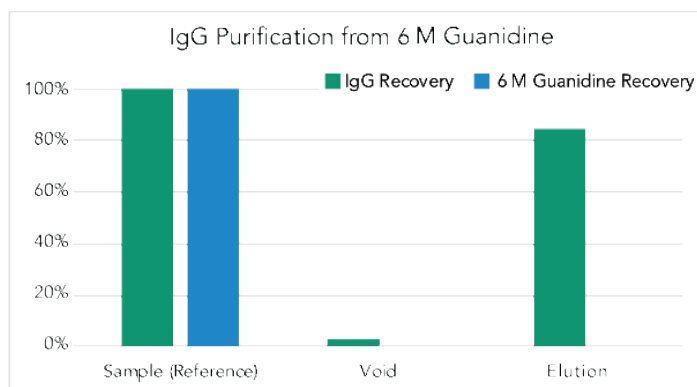
For automated INTip Swelling, SEC Tips aspirate buffer to swell the SEC resin. The sample containing 1 mg/mL IgG human protein antibody and 6 M guanidine is then added to the bed. The ALH then picks up the SEC Tip to dispense the sample into the SEC bed, displacing the swelling buffer and delivering it to a waste well plate. Once the 100  $\mu$ L sample has been dispersed across the SEC bed, 165  $\mu$ L of PBS is added to the top of the bed. The ALH then picks the SEC Tips back up and dispenses 65  $\mu$ L of void volume to the waste well plate. The protein is then eluted by dispensing the remaining 100  $\mu$ L of PBS into an elution plate. The piercing, automated INTip Swelling, and SEC method was performed in under 15 minutes. **Figure 3** shows the method schematic.



**Figure 3.** Schematic represents the workflow for INTip Swelling and purification of human IgG using DPX SEC Tips.

## RESULTS

This method provides greater than 85% recovery of human IgG protein antibody while removing the salt (as shown in **Figure 4**), demonstrating that this technology and method can be integrated into a large variety of protein isolation applications. With just a 100  $\mu$ L sample volume and 100  $\mu$ L elution volume, DPX SEC Tips minimize dilution of analytes of interest. Recovery can be further maximized while maintaining the undetected salt content in final solution by eluting with 50  $\mu$ L more. Although 6 M guanidine was used in this workflow, DPX SEC Tips can be utilized for common buffer exchanges up to a concentration of 8 M guanidine.



**Figure 4.** SEC Tips provide greater than 85% recovery of human IgG protein in 6 M guanidine, a high-salt content environment. Guanidine is not detected in target elution, and is still retained on the SEC bed, giving efficient buffer exchange and purified protein for downstream analysis.

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