Considerations for Automating the Purification of Monoclonal Antibodies on a Gilson Liquid Handler

TECHNICAL NOTE 0112

TONI R. HOFHINE, TOM STIGLIC, MARK MUNCEY – GILSON, INC.

OVERVIEW

Monoclonal antibody (mAb) use in biomedical research allows specific molecule binding to occur, aiding in subsequent identification and isolation for multiple disease treatments. For a single-step, small-scale purification with high purity results, monoclonal antibodies are purified using affinity columns. Conventional manual spin techniques or liquid chromatography systems are typical purification approaches, but it is also possible to purify antibodies with a simple liquid handler and syringe pump.

Scaled for low to medium throughput, a single-probe Gilson liquid handler can purify one antibody while a four-probe Gilson liquid handler can purify four antibodies simultaneously (Figures 1 and 2). With a Gilson liquid handling system, the sample is aspirated through the “bypass” side of a valve into a holding loop or transfer tubing (Figure 3a) before the valve is switched and dispenses the sample through the column (Figure 3b). Purification occurs during the elution step, when the probe moves to a collection tube. There are many considerations when moving from manual to automated mAb purification, and this technical note discusses some of the hardware and software considerations for maintaining sample integrity and reducing carryover when using a Gilson automated liquid handling system.

Figure 1
Gilson GX-241 Liquid Handling System

Figure 2
Gilson GX-274 Liquid Handling System

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MATERIALS & METHODS

Gilson Equipment

- Gilson GX-241 Liquid Handling System with one PEEK™ VALVEMATE® II 6-port (0.060”), 2-position valve (Figure 3)
- Gilson GX-274 Liquid Handling System with four PEEK VALVEMATE II 6-port (0.060”), 2-position valves (Figure 3); (one for each probe)
- Teflon coated probes
- TRILUTION® LH v 3.0 Liquid Handling Software

Tubing Selection

- 0.062” inner diameter (ID) Radel® R tubing (polyphenylsulfone) for transfer tubing (Figure 4)
- 0.062” ID Ultra-high purity PFA (HPFA+) (perfluoroalkoxy) tubing for transfer tubing (Figure 4)
- 0.062” ID PEEK™ (polyetheretherketone) tubing for small loop between open valve ports (pos 1)

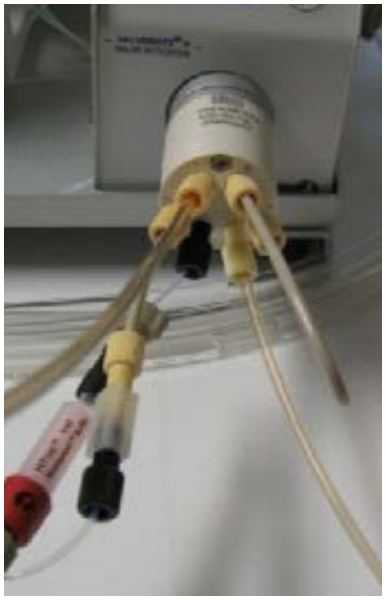

Figure 3
VALVEMATE II PEEK valve
- Pos 1: access to transfer tubing
- Pos 2: access to HiTrap™ column

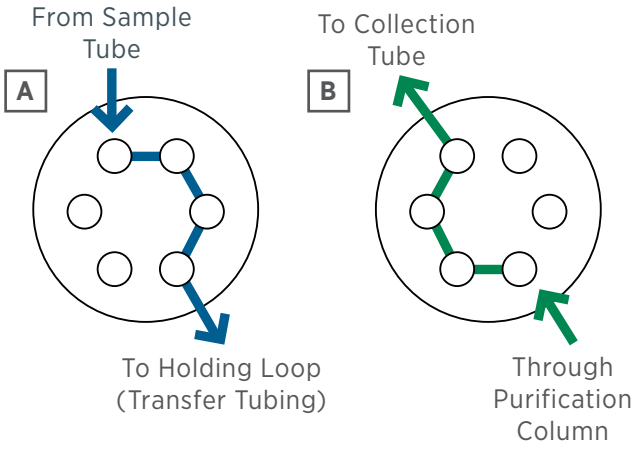

Figure 3
Position 1 Aspiration (A)
Position 2 Dispensing (B)

Figure 4
Transfer Tubing Radel R (left); - HPFA+ (right)

Sample

- Cell culture media (Miinimum Essential Medium Eagle) with 10% bovine serum

Purification Column

- GE Healthcare HiTrap MabSelect SuRe 1 mL (multiple columns can be used in series for scale-up applications)

Considerations for maintaining sample integrity and reducing carryover on an automated system are:

- Tubing and Fitting Selection
- Valve Material and Port ID
- Probe Material and ID
- Software: Flow Rate, Air Gap, Equilibration Time, Extra Volume, Rinse Volume

Tubing and Fitting Selection

The general accepted rule of thumb when working with biological samples, such as serum, cell culture, and protein, is to use an inert material that allows no sample to metal interaction. Keeping in mind that biological samples have higher viscosity than water, use larger ID tubing to improve volume accuracy. Transfer tubing on the Gilson system holds the sample volume prior to switching the VALVEMATE II valve to access the purification column.

- PEEK™ is generally an acceptable tubing choice; however, it is relatively opaque and rigid at inner diameters of 0.062". It is important to visually verify viscous biological samples traveling through the tubing for optimum software flow rates, equilibration times, etc. In this technical note, PEEK was used for a small loop between open ports in pos. 1 (Figures 3 and 3a).
- Radel® R (polyphenylsulfone) is similar to PEEK in chemical compatibility with biological samples, but has the added benefit of being translucent and highly flexible. In tests, the cell culture media with 10% bovine serum sample visibly stayed intact while traveling through the tubing. There were no visible air bubbles that formed (Figure 5) as the sample traveled into the purification column (one of the tips to avoid when using HiTrap™ columns). Rinsing between samples was more effective with Radel R tubing, removing concerns about carryover.
- Ultra-high purity PFA (perfluoroalkoxy) tubing was also selected as an alternative choice to PEEK for the same reasons as Radel R tubing. Often labeled as HPFA+, this tubing has an extra smooth inner wall, which was a good option for removing any potential sample interaction. Tests revealed that the air gap did not remain intact, showing multiple air bubbles visibly forming as the sample traveled through the tubing (Figure 6).

Figure 5
Radel R Tubing Showing Intact Air Gap

Figure 6
HPFA+ Tubing Showing Broken Air Gap

VALVE MATERIAL AND PORT ID

Valve material is equally important as tubing selection, because the sample travels through the valve to access either the transfer tubing that holds the sample volume during the aspiration step (pos. 1) or the purification column for the wash, equilibrate, load, wash, and elution steps (pos. 2). PEEK is a common rugged valve material, and a valve with 0.060" ports was chosen to reduce pressure and any potential for air bubbles forming as the sample travels between the 0.062" ID tubing and the 0.060" ID valve ports.

Probe Material and ID

Probe rigidity is important for long and repeated use to draw or aspirate the sample from the vial into the probe and then the transfer tubing. The probe material choice for this technical note was a Teflon® coated stainless steel Gilson probe. Teflon is inert for use with biological samples and cleans easily to remove carryover concerns between samples. A larger probe ID of 1.1 mm was chosen to allow for faster aspiration of the viscous sample for efficiency and accuracy.

Software

TRILUTION LH allows for selection of multiple parameters (flow rate, volume, air gap, equilibration time) from the Sample List in the Run screen through the use of variables written at the Method level. Because of this flexibility, the user can efficiently choose the variable parameters during R&D testing without rewriting the Methods (Figure 7). Once verified, these values can be entered into standard Methods for routine purification runs.

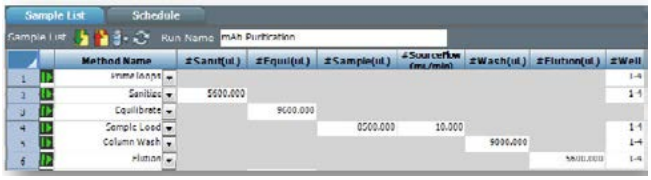

Figure 7
TRILUTION LH Sample List Showing Variable Columns for Efficient R&D

In this technical note, a TRILUTION LH flow rate of 10 mL/min (Figure 7) was determined as optimal for sample aspiration, and a flow rate of 2 mL/min (Figure 8) was optimal for dispensing or loading onto the purification column.

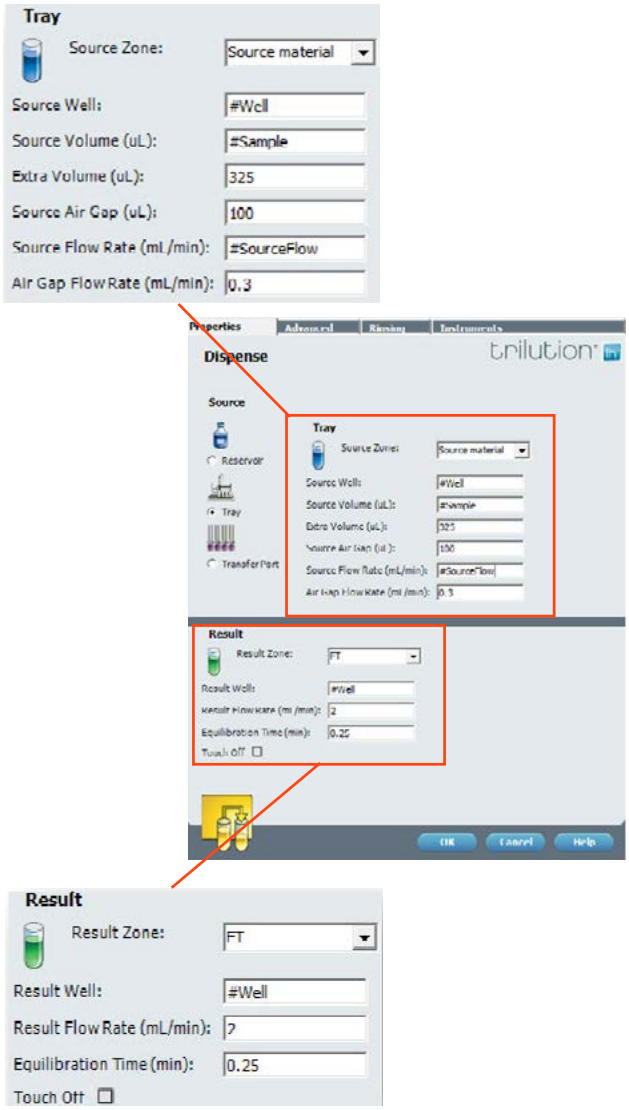

Figure 8
TRILUTION LH Dispense Task

The extra volume and equilibration times are used together to maintain accuracy with a viscous sample. A smaller equilibration time is used with an optimal extra volume to keep the Teflon coated probe in the sample vial while the viscous sample continues to travel in the transfer tubing after the aspiration and dispense steps. Simple volumetric tests verify if the combination chosen provides accurate volume transfer.

Separating the sample from the water reservoir system fluid (red dot in Figure 9) in the transfer tubing was an air gap of 100 uL. Air gap size was chosen based on the tubing diameter, speed of aspiration, and viscosity of the biological sample. The example Dispense TRILUTION LH Task in Figure 8 shows the simple interface at the Method level.

Figure 9
Reservoir System Fluid

A final consideration is adequate rinsing between samples. With the Radel® R tubing, this important process is streamlined. In tests, rinsing was performed at the end of each Method step using the Rinse Probes Task (Figure 10). Rinsing volumes can be tested by running a sample with an internal standard (IS), followed by a control to compare if consistent IS values are obtained. TRILUTION LH has the flexibility to allow for multiple rinses from a variety of userselected solvents, as well as the flexibility to add or include rinsing wherever required within a Method.

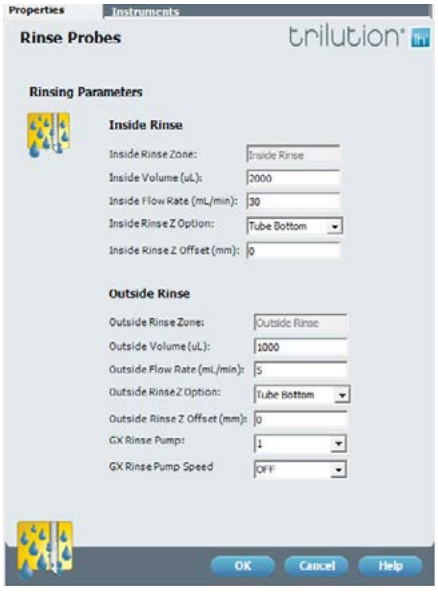

Figure 10
TRILUTION LH Rinse Task

Notice

This application note has been produced and edited using information that was available at the time of publication. This application note is subject to revision without prior notice.

PIPETMAX is a general liquid handing device to be used by trained scientific personnel in general laboratories, designed but not limited to the following uses:

Prevention of cross contamination:
- Specific filters
- Single-use disposable pipette tips

Linked to the material compatibility with biological samples and reagents:
- Choice of raw materials
- Sterilization processes for single-use consumables

Improvement in the result of measurements:
- Accuracy of volumes
- Improve reproducibility through automation