

## APPLICATION NOTE

# Alternative Method for mAb Purification: Rapid Purification without a Liquid Chromatography Unit using a GORE™ Protein Capture Device

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## Objective

To assess the speed, purity, and yield performance of the GORE Protein Capture Device with Protein A to purify antibodies using alternative methods to Liquid Chromatography (LC) units.

## Background

Liquid chromatography units provide exceptional process monitoring controls and automation for the purification of biomolecules including monoclonal antibodies. However, many labs perform purifications without an LC unit, using methods such as a syringe or a peristaltic pump system. The GORE Protein Capture Device with Protein A can be used as demonstrated in three different manual/semi-automated operations to rapidly purify monoclonal antibodies without an LC unit.

### Methods Evaluated:

1. Manual Syringe Purification
2. Syringe Pump Purification
3. Peristaltic Pump Purification

### Materials:

Table 1. Materials required to execute the methods outlined

Materials	Part Number
1.0 mL GORE Protein Capture Device	PROA101
NORM-JECT 30 mL Luer Syringe	4830003000
GE; 1/16" male/luer female	28-9858-12
Nordson Medical; Male luer/200 series barb, 1/16"	MTLL210-6005
15 mL Falcon Centrifuge Tubes	352096
MasterFlex Tubing (Size 13)	06475-13
New Era Pump Systems Inc. Syringe Pump	NE-300
MasterFlex Console Drive	77521-40
MasterFlex Easy-Load II	77201-60

## Procedure

The same buffers were used for consistency between the three purification methods. The purification process including buffers and wash are shown in Table 2. Purified monoclonal antibody (IgG<sub>1</sub>) was used for each method of purification to demonstrate capability and capture yield values with minimal equipment requirements. A UV/Vis spectrophotometer was used to evaluate antibody concentrations, all evaluated at an absorbance of 280nm.

Table 2. Buffers used for each purification method

Buffer	Column Volumes (CV)	Volume (mL)	Step Process
DI H2O	10 CV	10 mL	Rinse
PBS*	10 CV	10 mL	Equilibrate
MAB	10 CV	10 mL	Load
PBS	10 CV	10 mL	Wash
Citrate	4 CV	4.0 mL	Elution
Citrate	6 CV	6.0 mL	Wash
PBS	5 CV	5.0 mL	pH Adjustment
NaOH	10 CV	10 mL	CIP
PBS	8 CV	8.0 mL	pH Adjustment
DI H2O	5 CV	5.0 mL	Salt Removal
20% Ethanol	5 CV	5.0 mL	Storage

\*PBS – Phosphate-buffered saline  
CIP – Clean-in-place



## Method 1: Manual Syringe Purification



Figure 1. Manual purification using a 30 mL syringe attached to a 1.0 mL GORE™ Protein Capture Device

Method 1 used a 30 mL luer adapted syringe attached to a 1.0 mL GORE Protein capture device using a GE, 1/16" male/luer female fitting (Figure 1). The buffer order and volume dispensed per step followed the recipe outlined in Table 2. The flow-through byproducts from the monoclonal antibody loading step were collected to evaluate any unbound antibody that passed through the device. A nominal dynamic binding capacity (DBC) of 30 mg/mL was assumed for loading, and the device was loaded to ~80-90% of maximum DBC.

Fractions of 4.0 mL were collected during the elution step and neutralized using 1.0 mL of 1M Tris, pH 9.0. The CIP step for this process followed Table 2 with a pause for 3-5 minutes after loading the first 5 CV of 0.1M sodium hydroxide to ensure sanitization of the device. A total of three purifications (bind-elute) were performed for this method using the same device, with an average total time of 28 minutes and 40 seconds, not including the CIP hold time.

## Method 2: Syringe Pump Purification

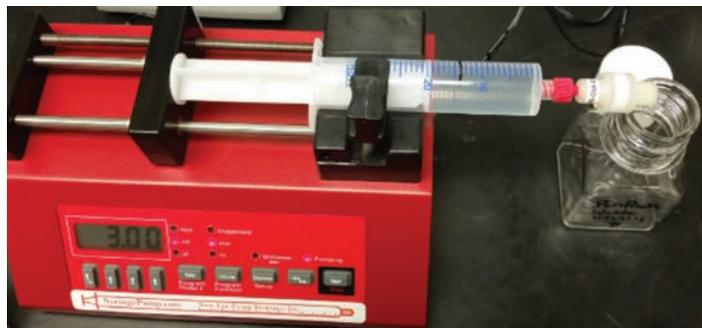


Figure 2. Semi-automated purification using a syringe pump and a 30 mL syringe attached to a 1.0 mL GORE Protein Capture Device

Purification Method 2 follows the same steps and procedures as the manual syringe purification with the aid of a syringe pump (Figure 2), operated at 20 seconds residence time for the GORE Protein Capture Device (3.0 mL/min; see operating instructions). Loading and elution steps as well as neutralization of fractions for the purification followed the steps in the first method. The same GORE device was used for both Method 1 and Method 2. Total time for this semi-automated purification method was 29 minutes and 20 seconds, not including the CIP hold time.

## Method 3: Peristaltic Pump Purification Results



Figure 3. Semi-automated purification method using a peristaltic pump, attached to a 1.0 mL GORE™ Protein Capture Device

Purification Method 3 followed the same steps and procedures as the syringe purification methods with an additional priming step between each buffer/antibody transfer (Figure 3). This method also used a second fitting (Nordson Medical; Male luer/200 series barb, 1/16"; see Table 1) to attach the pump tubing to the GE fitting. The priming step for each solution transfer was performed by capping the outlet of the GORE Protein Capture Device, detaching the fitting from the device, priming the new solution through the lines, then reattaching the device to the fitting and removing the cap on the outlet side to start the next step. All other steps were the same as the first two methods. The total time for this semi-automated purification method was 28 minutes and 37 seconds, not including the CIP hold time.

Results for the three methods outlined above can be seen in Table 3. All purification methods fell within the specifications for the GORE Protein Capture Device.

Table 3. Purification Results for three methods with GORE Protein Capture Device

Method	MAb Feed Conc. (mg/mL)	MAb Loaded (mg)	Elution Pool Conc. (mg/mL)	Yield (%)	Leached Protein A (ppm)
Method 1: Manual Syringe Purification	2.66	26.6	5.19	97.5	13.7
Method 2: Syringe Pump Purification	2.64	26.4	5.07	97.9	8.1
Method 3: Peristaltic Pump Purification	2.68	26.8	5.25	98.1	7.5

## Conclusion

The three purification methods outlined in this application note provide alternative options to successfully purify a monoclonal antibody in under 30 minutes with yields greater than 95%. These methods provide the ability to perform affinity purifications without the use of an LC unit. Though these methods are feasible options for purification, individualized feed stocks purification results will vary. The method and buffers should be optimized based on the target antibody and specific feed stock. Further optimization of the protocol may be explored to fit the needs of a given lab.

## Gore PharmBIO Products

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