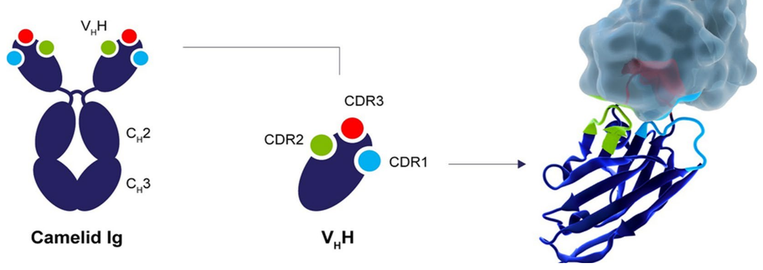
High Throughput Screening of affinity chromatography for new modalities: case studies with GoPure 96-well screening plates

David Yang[1], Alejandro Becerra[1], Chiu Lau-Barre[2], Ben Waite[1], Al de Leon[1]
[1]Thermo Fisher Scientific, Bedford, MA, USA, [2]Thermo Fisher Scientific, Carlsbad, CA, USA

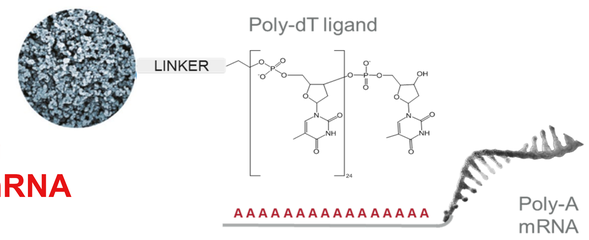
Introduction

POROS™, CaptureSelect™ and Oligo (dT)25 resins have been successfully used for process purification for adeno-associated virus (AAV), antibodies and mRNA. CaptureSelect™ affinity ligands are camelid single domain V_HH fragments of ~15 kDa (illustration below), the smallest antigen binding ligand allows its binding at difficult-reached epitopes of target AAV, antibody and protein with high affinity and specificity.

CaptureSelect Affinity Ligand for FcXP, AAVX and AAV9 resins



POROS Oligo (dT)25 affinity resin binding to mRNA



The GoPure™ 96-well screening plates filled with CaptureSelect™ and POROS™ affinity resins provide a convenient and efficient platform for screening multiple affinity chromatographic conditions in parallel. By distributing the resins consistently in each well of the 96-well plate format, researchers can conduct fast screenings of different experimental conditions. The case studies conducted using the GoPure 96-well screening plates on AAV9, human plasma IgG, and mRNA feeds demonstrate the effectiveness of this approach in optimizing affinity chromatography conditions.

Materials and Methods

- POROS™ GoPure™ Oligo (dT)25, POROS™ GoPure™ AAV9, POROS™ GoPure™ AAVX, and CaptureSelect™ GoPure™ FcXP 96-well screening plates (Thermo Fisher Scientific) containing prefilled 20 µL resin/well were used.
- Samples (e.g., mRNA, plasma, and AAV serotype) and buffers were mixed with resins in the screening plates on orbital microplate shaker at 1100-1400 RPM.
- For incubation both top and bottom of the plates were sealed using a strong adhesive plate seal (Fisher scientific AB-0558) to prevent leakage of the samples/buffers from the plates.
- Flow-through, wash and elution samples were collected in 96-deep well plates by centrifuge (1000-1500 x g for 2 min), alternatively by vacuum manifold for 96-well filter plates.
- Addition of samples and buffers were handled by liquid handling system and/or multichannel pipettes.

Reproducibility of GoPure 96-Well Screening Plates

Experiment: Binding capacity of oligo (dA)-40mer on POROS Oligo (dT)25 affinity resin in GoPure 96-well screening plate was used to evaluate the plate-to-plate and well-to-well reproducibility. Eluted oligo dA was measured by UV absorbance at 260nm and quantitated using an oligo (dA)-40mer standard curve.

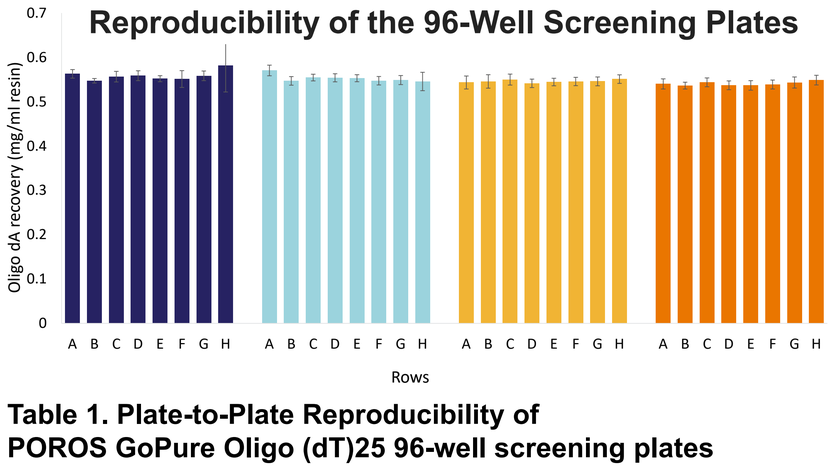


Table 1. Plate-to-Plate Reproducibility of POROS GoPure Oligo (dT)25 96-well screening plates

	Binding capacity (mg/ml resin) on average and %RSD for each of the plates				
	Plate #1 (n=96)	Plate #2 (n=96)	Plate #3 (n=96)	Plate #4 (n=96)	Average (4 plates)
Capacity	0.56	0.55	0.55	0.54	0.55
%RSD	2.06	2.06	2.18	2.01	2.08

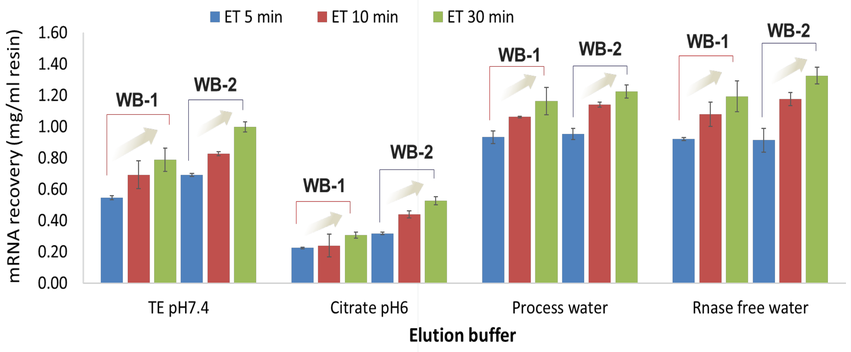
- Using oligo (dA) binding to oligo (dT)25 affinity resin in GoPure Oligo (dT)25 96-well plates we have successfully demonstrated highly consistency of the GoPure 96-well screening plates.

mRNA purification screening with POROS Oligo (dT)25 96-well plates

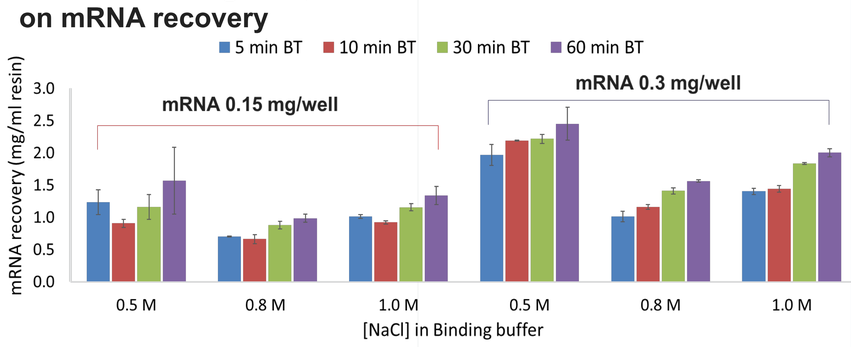
Spin and equilibration	Load mRNA (4000nt)	Plate seal and mix	Flow-through and wash	Elution	A260nm measurement
mRNA (4000nt) load	Binding time	Binding buffer	Wash buffer	Elution buffer	Elution time
0.15 mg/well	5 min	0.5 M NaCl in TE Buffer	0.1M NaCl in TE Buffer	TE buffer	5 min
0.3 mg/well	10 min	0.8 M NaCl in TE Buffer	0.2M NaCl in TE Buffer	10mM sodium citrate, pH = 6.0	10 min
	30 min	1.0 M NaCl in TE Buffer		Process water	30 min
	60 min			RNase-free water	

0.5 mg/ml and 1.0 mg/ml mRNA | 300 µL/well Sample volume

Effect of elution time and buffers on mRNA recovery



Effect of binding time, salt concentration and loaded amount on mRNA recovery



- mRNA recovery was improved by (1) an intermediate wash with 0.2M NaCl, (2) extended elution time, (3) eluted by water, Including 0.5M NaCl in binding buffer, extended binding time and a sufficient amount of sample loaded also improved the mRNA recovery.
- Operating parameters was established with the screening experiments using POROS oligo (dT)25 96-well plates.

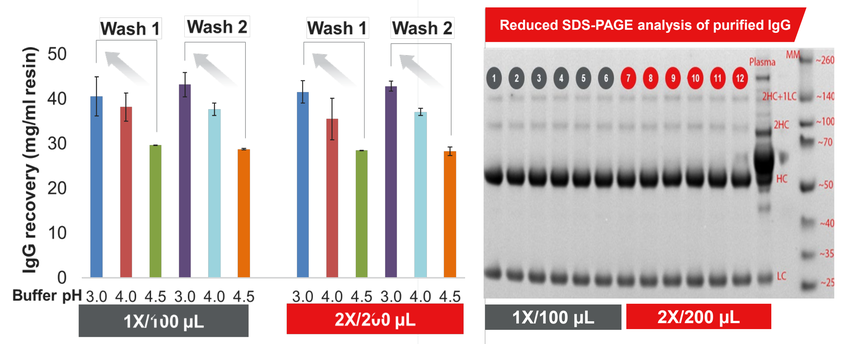
Human plasma IgG purification screening with CaptureSelect FcXP 96-well plates

Spin and equilibration	Load human plasma	Plate seal and mix	Flow-through and wash	Elution	A280nm measurement
Plasma dilution		Wash buffer		Elution buffer	
Diluted 1x		50 mM Tris-HCl, pH 7.4, 1M NaCl		50 mM acetic acid, pH 3.0	
Diluted 2x		50mM Tris-HCl, pH 9.0, 1M NaCl		50 mM acetic acid, pH 4.0	
Diluted 4x				50 mM acetic acid, pH 4.5	

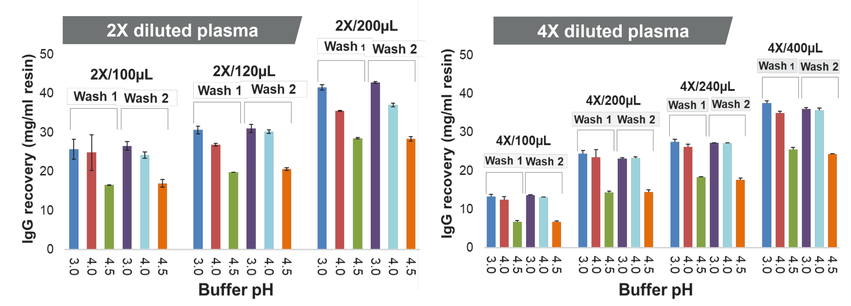
Dilution in equilibration buffer: 50 mM Tris-HCl, pH 7.4, 0.125 M NaCl

Eluted IgG was measured by UV absorbance at 280nm. | IgG purity was analyzed by reduced SDS-PAGE

Effect of plasma dilution and pH on IgG recovery and purity



Effect of loaded amount, wash and elution pH on IgG recovery



- Dilution of the plasma and intermediate washes showed little effect on the purification recovery and purity.
- IgG recovery increased with increasing the plasma amount loaded and by elution at a range of pH 3.0 to 4.0.

AAV9/AAV6 purification screening with POROS AAV9/AAVX 96-well plates

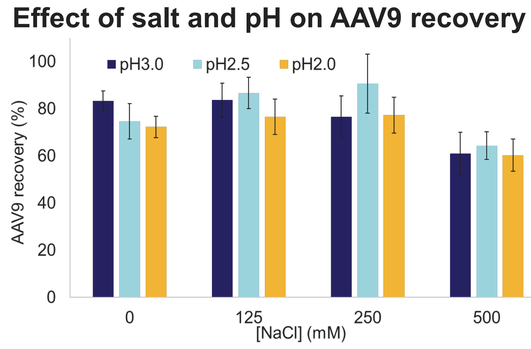
Spin and equilibration	Sample: AAV9/AAV6	Plate seal and mix	Flow-through and wash	Elution	AAV9 ELISA analysis

0.1 M Glycine elution buffers of various pH, NaCl concentration and additive (18 different buffers)

pH	[NaCl]	Additive
2.0	0 mM	Arginine (0.3M and 0.5M)
2.5	125 mM	$MgCl_2$ (0.5M and 1.0M)
3.0	250 mM	Propylene Glycol (20% and 40%)
	500 mM	

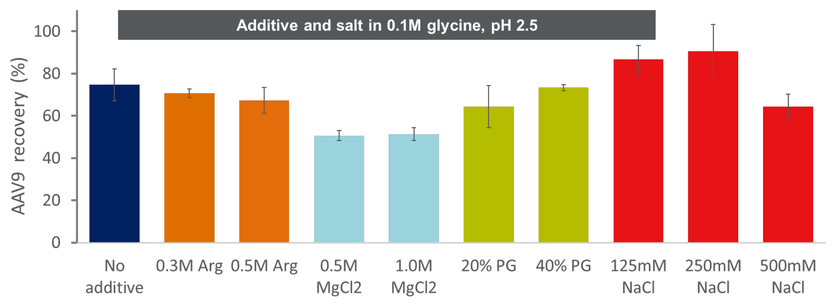
AAV9 conc: 2.62 E12 cp/ml
AAV6 conc: 1.30 E13 cp/ml
Load 400 µL per well | AAV9 sample and eluate titers were analyzed using an AAV9 Xpress ELISA | Each buffer condition tested in triplicates on the 96-well plates

Effect of salt and pH on AAV9 recovery on AAV9 resin



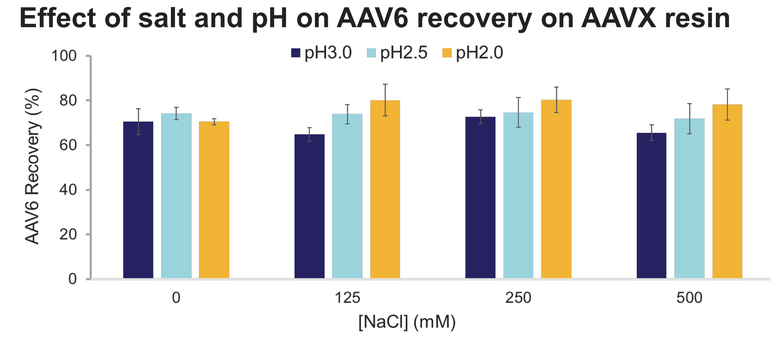
Glycine buffer provides efficient elution over the pH range 2-3, and effect of pH elution is associated with NaCl concentration. AAV9 recovery was improved with 0.1M glycine pH 2.5 with NaCl concentration 125-250mM

Effect of buffer additives on AAV9 recovery on AAV9 resin



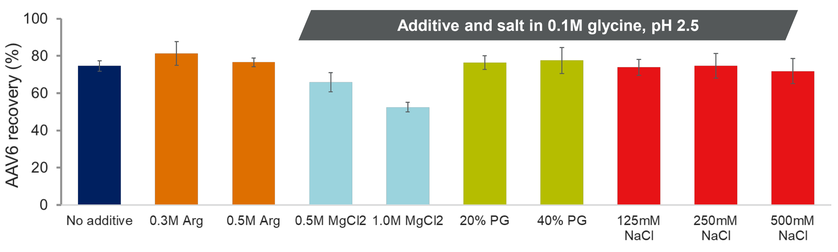
- 250mM NaCl was more effective than the additives of arginine, MgCl2 and propylene glycol in the glycine elution buffer on AAV9 elution from AAV9 resin.

Effect of salt and pH on AAV6 recovery on AAVX resin



- The glycine buffer with or without salt showed similar elution recovery. AAV6 recovery on AAVX resin was improved with 0.1M glycine buffer containing125-250mM NaCl at pH 2.0.

Effect of buffer additives on AAV6 recovery on AAVX resin



- AAV6 recovery on AAVX resin can be improved by addition of arginine and propylene glycol in the glycine elution buffer.

Conclusion

- ✓ High reproducibility and consistency were demonstrated for GoPure™ 96-well screening plates prefilled with POROS™ and CaptureSelect™ affinity resins.
- ✓ The GoPure™ 96-well screening plates allowed rapid screening of chromatographic experimental conditions for affinity purification of mRNA, AAV and human plasma IgG.
- ✓ The results from these experiments can be used to guide future column experiments to aid in expediting process development of these newer therapeutic modalities.

Trademarks/licensing

© 2024 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.

POROS resins: Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only. CaptureSelect ligands and resins: For Research Use or Further Manufacturing. Not for diagnostic use or direct administration in humans or animals.