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

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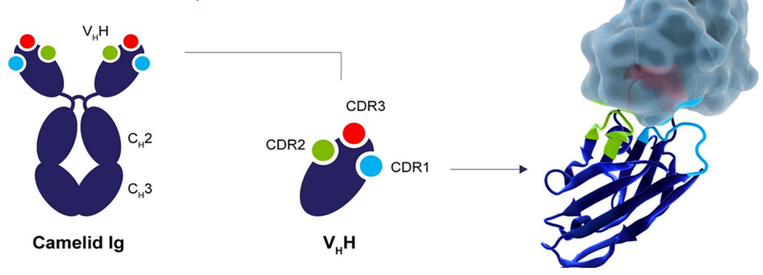
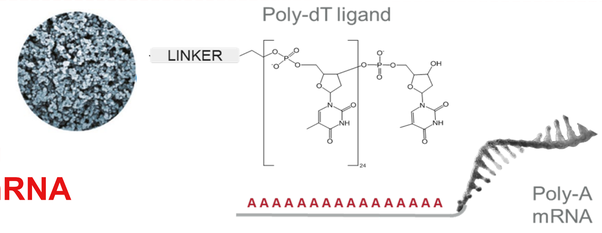
Introduction

Thermo Scientific ™ POROS™ resins, Thermo Scientific™ CaptureSelect™ resins, and Thermo Scientific™ POROS™ Oligo (dT)25 resin 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 Thermo Scientific™ 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

- Thermo Scientific™ POROS™ GoPure™ Oligo (dT)25, Thermo Scientific™ POROS™ GoPure™ AAV9, Thermo Scientific™ POROS™ GoPure™ AAVX, and Thermo Scientific™ CaptureSelect™ GoPure™ FcXP 96-well screening plates 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.

Results:

Binding capacity (mg/ml resin) on average and %RSD (n=12) for each row of the plates								
Row#	Plate #1	*%RSD (1)*	Plate #2	*%RSD (2)*	Plate #3	*%RSD (3)*	Plate #4	*%RSD (4)*
A	0.56	*1.48*	0.57	*2.42*	0.54	*2.82*	0.54	*2.12*
B	0.55	*0.96*	0.55	*1.74*	0.55	*2.90*	0.54	*1.42*
C	0.56	*2.21*	0.55	*1.37*	0.55	*2.36*	0.54	*1.82*
D	0.56	*2.10*	0.55	*1.71*	0.54	*1.86*	0.54	*1.85*
E	0.55	*1.33*	0.55	*1.43*	0.54	*1.63*	0.54	*2.00*
F	0.55	*1.53*	0.55	*1.77*	0.55	*1.79*	0.54	*1.86*
G	0.56	*2.02*	0.55	*1.86*	0.55	*1.92*	0.54	*2.30*
H	0.57	*1.67*	0.56	*2.02*	0.55	*1.85*	0.55	*1.99*

Table 2. 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 screening plates we have successfully demonstrated highly consistency of the GoPure 96-well screening plates.

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

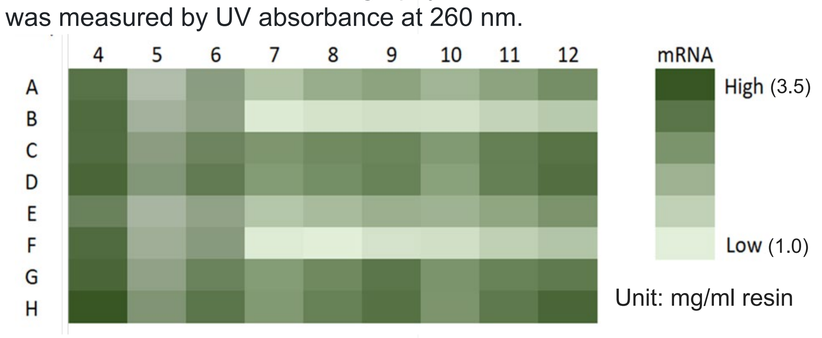
Chromatography conditions

Binding time (BT)	5 min 10 min 30 min 60 min
Binding buffer	B-1: 0.5M NaCl in TE buffer (10 mM Tris, 1mM EDTA, pH 7.4) B-2: 0.8 M NaCl in TE buffer B-3: 1.0M NaCl in TE buffer
Wash buffer	W-1: 0.1 M NaCl in TE buffer W-2: 0.2 M NaCl in TE buffer
Elution buffers (EB)	EB-1: TE buffer EB-2: 10 mM sodium citrate buffer, pH 6 EB-3: Process water EB-4: RNase free water
Elution time (ET)	5 min 10 min 30 min
mRNA (4000nt) loaded per well	0.3 mg for binding study 0.15 mg for wash and elution studies

Plate layout

		BT (minutes)	4	5	6	Elution Buffer	7	8	9	10	11	12
			Binding buffer NaCl concentration				Elution time (minutes)			Elution time (minutes)		
T1	A	5	0.5 M	0.8 M	1.0 M	EB-1	5	10	30	5	10	30
	B	10	0.5 M	0.8 M	1.0 M	EB-2	5	10	30	5	10	30
	C	30	0.5 M	0.8 M	1.0 M	EB-3	5	10	30	5	10	30
	D	60	0.5 M	0.8 M	1.0 M	EB-4	5	10	30	5	10	30
T2	E	5	0.5 M	0.8 M	1.0 M	EB-1	5	10	30	5	10	30
	F	10	0.5 M	0.8 M	1.0 M	EB-2	5	10	30	5	10	30
	G	30	0.5 M	0.8 M	1.0 M	EB-3	5	10	30	5	10	30
	H	60	0.5 M	0.8 M	1.0 M	EB-4	5	10	30	5	10	30
							W-1: 0.1M NaCl			W-2: 0.2M NaCl		

Results: The heatmap illustrates recovery of mRNA (4000nt) upon elution under various chromatography conditions. Amounts of mRNA was measured by UV absorbance at 260 nm.



- Optimized binding condition for the mid-size mRNA on POROS Oligo (dT)25 resin is to use 0.5 M NaCl in TE buffer with extended binding time of 30 minutes or 60 minutes.
- Optimized recovery is to use water elution at extended elution time of 30 minutes, and with intermediate wash using 0.2 M NaCl in TE buffer. The overall results also demonstrated high well-to-well reproducibility.

Human plasma IgG purification screening on CaptureSelect GoPure FcXP 96-well screening plates

Chromatography conditions

Human plasma sample	Wash buffer	Elution buffer	Stripping buffer
PS-1X: Plasma (Total IgG: 17.7 mg/ml)	W-1: 50 mM Tris-HCl, pH 7.4, 1 M NaCl	EB-1: 50 mM acetic acid, pH 3.0	0.1 M phosphoric acid, pH 2
PS-2X: Plasma 2X dilution (Total IgG: 8.85 mg/ml)	W-2: 50 mM Tris-HCl, pH 9.0, 1 M NaCl	EB-2: 50 mM acetic acid, pH 4.0	
PS-4X: Plasma 4X dilution (Total IgG: 4.43 mg/ml)		EB-3: 50 mM acetic acid, pH 4.5	

Experiment: IgG concentration in human plasma was determined by IgG (Total) ELISA (Thermo Fisher Scientific). Eluted IgG was measured by UV absorbance at 280nm.

Results:

Fig. 1. Loaded with PS and PS-2X and washed by W-2

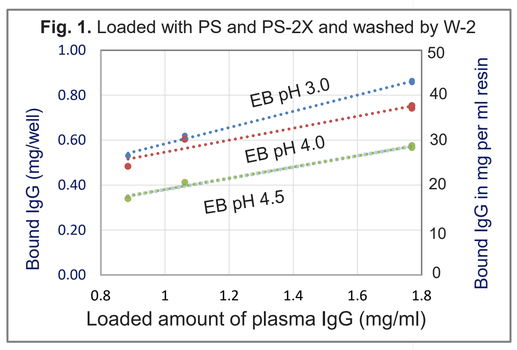


Fig. 3. Bound IgG vs dilution and wash buffer (Load = 1.8mg IgG)

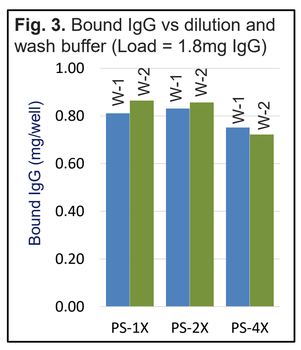
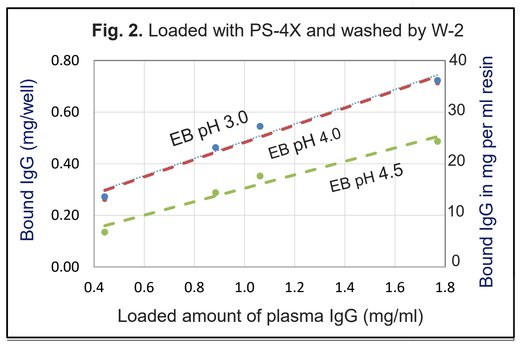


Fig. 2. Loaded with PS-4X and washed by W-2



- Optimized plasma IgG purification condition is to use 2X diluted or no-diluted plasma at a plasma to resin volume ratio of 5:1 with 1 M NaCl pH 9.0 intermediate wash, elution at pH 3.0.
- Binding capacity increased linearly as an increase in loaded amount of feed plasma IgG within a range of dynamic binding capacity of 40-50 mg/ml for Thermo Scientific™ CaptureSelect™ FcXP affinity resin.

AAV9 purification screening with GoPure AAV9/AAVX 96-well screening plates

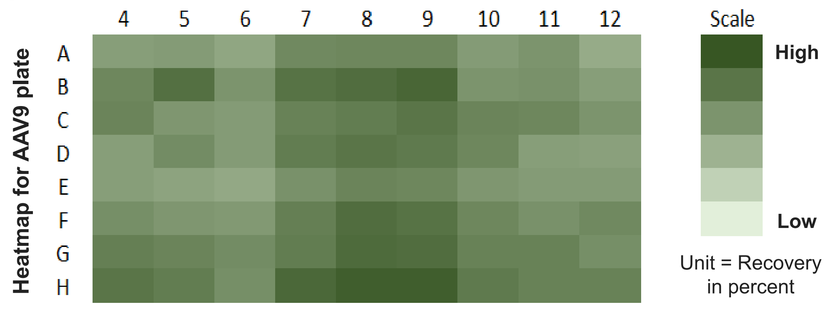
Chromatography conditions

AAV9 clarified lysate samples	Elution buffers	Binding time
S-1: lysate in lysis buffer partially treated by nuclease (2.63E+11 capsids/ml)	EB-1: 0.1 M glycine, pH 3.0, 0.01% PF-68	15 min
S-2: lysate in lysis buffer treated by additional nuclease (2.46E+11 capsids/ml)	EB-2: 0.1 M glycine, pH 2.5, 0.01% PF-68	30 min
S-3: Lysate treated by additional nuclease and in the presence of 0.1M NaCl in Tris buffer pH7.5, 0.01% PF-68 (2.08E+11 capsids/ml)	EB-3: 0.1 M glycine, pH 2.0, 0.01% PF-68 EB-4: 0.1 M glycine, pH 2.5, 0.3 M arginine, 0.01% PF-68	60 min
S-4: Lysate treated by additional nuclease and 0.5M NaCl in Tris buffer pH 7.5, 0.01% PF-68 (2.23E+11 capsids/ml)	EB-5: 0.1 M Citric acid, pH 3.0, 0.01% PF-68 EB-6: 0.1 M Citric acid, pH 2.5, 0.01% PF-68	90 min

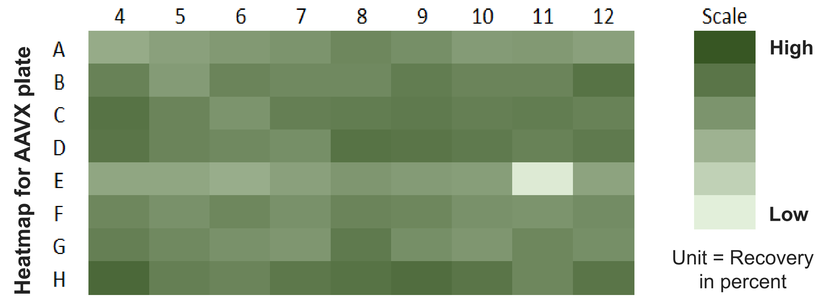
Plate layout

	4	5	6	7	8	9	10	11	12
	EB-1	EB-2	EB-3	EB-4	EB-5	EB-6	EB-1	EB-2	EB-3
	90 min						60 min		
A	S-1	S-1	S-1	S-1	S-1	S-1	S-1	S-1	S-1
B	S-2	S-2	S-2	S-2	S-2	S-2	S-2	S-2	S-2
C	S-3	S-3	S-3	S-3	S-3	S-3	S-3	S-3	S-3
D	S-4	S-4	S-4	S-4	S-4	S-4	S-4	S-4	S-4
	30 min						15 min		
E	S-1	S-1	S-1	S-1	S-1	S-1	S-1	S-1	S-1
F	S-2	S-2	S-2	S-2	S-2	S-2	S-2	S-2	S-2
G	S-3	S-3	S-3	S-3	S-3	S-3	S-3	S-3	S-3
H	S-4	S-4	S-4	S-4	S-4	S-4	S-4	S-4	S-4

Results for AAV9 resin: The heatmap illustrates elution recovery of AAV9 serotype from Thermo Scientific™ POROS™ CaptureSelect AAV9 resin under various chromatography conditions. AAV9 concentration was measured by AAV9 titer ELISA, and recovery was derived by the amounts eluted/loaded.



Results for AAVX resin: The heatmap illustrates elution recovery of AAV9 serotype on Thermo Scientific™ POROS™ CaptureSelect AAVX resin under the various chromatography conditions. Concentration measured similarly as AAV9 resin plate



- An effective binding condition for AAV9 on both AAV9 and AAVX resins was found to have feed treated by nuclease, with 0.5 M NaCl in the sample solution, and a binding time of 30 minutes. Various chromatographic conditions on the affinity binding can be screened using GoPure AAV9 or AAVX 96-well screening plates.
- While the elution buffers screened showed no significant effect on AAV9 elution on AAVX resin, the citric acid buffers showed more effect than glycine buffer without arginine on AAV9 elution on the AAV9 resin. Including 0.3 M arginine in the glycine buffers significantly improved the AAV9 recovery from AAV9 affinity resin.

Conclusion

- The reproducibility and consistency of the GoPure 96-well screening plates were demonstrated with POROS and CaptureSelect affinity resins.
- The GoPure 96-well screening plates allowed rapid screening of binding and elution conditions for affinity chromatographic 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.

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