Developing new affinity options for albumin and albumin fusion proteins

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AVITIDE
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Exquisite purity and high caustic stability of AVIPure® Albumin resins provide new options for large scale purification

- Higher purity than current processes
- NaOH stability enabling >>100 reuse cycles
- Consistent yield with multiple elution regimes
- High DBC

AVIPure Albumin resins

Both AVIPure Albumin and AVIPure Albumin Fusion resins selectively bind human albumin with very high affinity.

The AVIPure Albumin resin is optimized for high capacity and selectivity for recombinant and serum-derived albumin, while the AVIPure Albumin Fusion resin is ideal for larger constructs that utilize albumin as a fusion partner.

The AVIPure Albumin Fusion affinity ligand is designed to minimize interference with fusion partners based on the location of the binding site (see Figure 1).

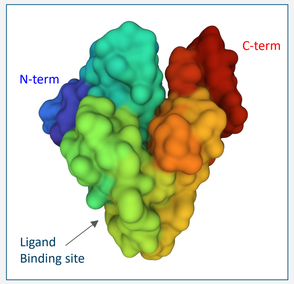

Figure 1: Human albumin structure

Table 1: Performance parameters for AVIPure resins

Parameter	AVIPure Albumin	AVIPure Albumin Fusion
Binding Capacity (g/L resin)	>40 at pH 7.4 >50 at pH 5	~25 Varies w/ size of fusion partner
Residence Time	4 min	
Elution Conditions	1) 15% hexanediol, 0.5 M proline, pH 9 2) 200 mM sodium octanoate, 1 M NaCl, pH 8 3) pH 3	
CIP Conditions	0.5 M NaOH	
Step Yield	>90%	
Residual HCP ng/mg (ppm)	<2,000 ppm	<1,000 ppm
Residual DNA ng/mg (ppm)	<40 ppm (PicoGreen), <2 ppb (qPCR)	
Number of Reuses	>>100	

AVIPure Albumin achieves >40 g/L DBC

Breakthrough curves in Figure 2 demonstrate the capacity of AVIPure Albumin relative to other affinity options. The capacity of AVIPure Albumin is dependent on the load pH; the resin achieves 65 g/L capacity when loaded at pH 5 and further increases, up to 18%, at longer (8-minute) residence times.

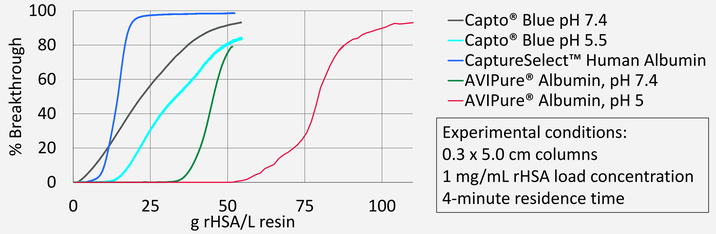

Figure 2: Breakthrough curves for recombinant human albumin from serum (rHSA)

>>100 Reuse cycles with 0.5 M NaOH

Reuse of the resin was demonstrated with DBC experiments using a 0.3 x 5.0 cm column after exposure to 0.5 M NaOH for up to 72 hours. The DBC is unchanged after 48 hours exposure (see Figure 3). High caustic stability offers several CIP options listed in Table 2.

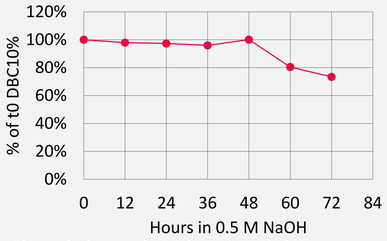

Figure 3: Change in $DBC_{10\%}$ after NaOH exposure

Table 2: Clean-In-Place (CIP) regimen options for AVIPure Albumin resins

CIP Agent	Cycles	CIP Regimen
0.5 M NaOH	90	30 minutes/cycle
0.25 M NaOH	180	30 minutes/cycle
0.1 M NaOH	900	15 minutes/cycle

Pure rHSA (from Sigma) was spiked into a *Pichia pastoris* fermentation supernatant to 4 mg/mL and loaded to 25 g/L_{resin} using a 0.3 x 5.0 cm column and 4-minute residence time. The purity of the AVIPure Albumin elution pool is improved over that of the pure rHSA by SDS-PAGE (Figure 4) and equivalent by HP-SEC (data not shown).

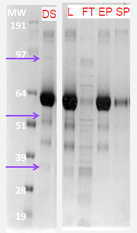

Figure 4: SDS-PAGE analysis of AVIPure Albumin eluates from *Pichia pastoris* fermentation

Reduced SDS-PAGE
DS: 2 µg rHSA
L: rHSA-spiked load (2 µg rHSA)
FT: flow through pool
EP: elution pool (2.1 µg rHSA)
SP: strip pool

Purple arrows indicate impurity bands present in rHSA (Sigma) and the AVIPure Albumin resin flow through, but not in the elution pool

AVIPure Albumin Fusion capacity varies with size

The size of the fusion partner will affect the overall binding capacity of the resin. Table 3 gives the performance parameters for several fusion partner proteins of varying size.

Table 3: Performance of AVIPure Ablumin Fusion resin with various targets

Target	Fusion	Target size by SEC (kDa)	AVIPure AF K_D (nM)	AVIPure AF SBC (g/L_{resin})	AVIPure AF $DBC_{10\%}$ (g/L_{resin})	Capto Blue $DBC_{10\%}$ at pH 5.5 (g/L_{resin})	CaptureSelect $DBC_{10\%}$ (g/L_{resin})
HSA	-	66	3	32	30	18	10
AF#1	N-term	300	<1	21	11	Not tested	Not tested
AF#2	C-term	120	2	29	Not tested	Not tested	Not tested
AF#3	C-term	120	<1	26	Not tested	Not tested	Not tested
AF#4	C-term	150	1	30	29	17	9
AF#5	C-term	76	<1	35	24	Not tested	Not tested

Resin cycling with clarified lysate of AF#4 shows robust resin performance over a total exposure time of 24 hours to 0.5 M NaOH (see Figure 5 and Figure 6).

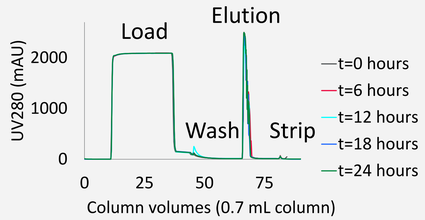

Figure 5: Overlaid chromatograms of AVIPure Albumin Fusion cycles with AF#4 feed

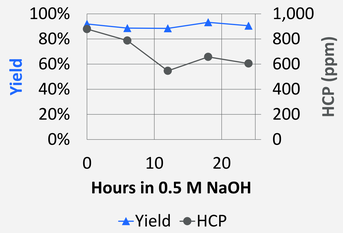

Figure 6: Yield and purity for AVIPure Albumin Fusion cycling study with AF#4

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