Purification of rHSA from Recombinant Rice Extracts with AVIPure® Albumin Affinity Resin

Application Note

Introduction

AVIPure® Albumin is a high-capacity, hydroxide stable affinity resin for the purification of albumin and albumin fusion proteins from a variety of feed streams, including mammalian, yeast, and plant sources.

AVIPure Albumin has a high capacity for recombinant albumin in rice extracts. The capacity increases with decreasing pH, up to 70 g albumin/L_{resin} at pH 4.5. The resin offers flexible elution options, with both pH 3 and pH 8 elution buffers providing high purity and yield.

AVIPure Albumin Affinity Resin tolerates exposure to 0.5 M NaOH for 48 hours, providing an efficient, cost-effective solution for purification of albumin and albumin fusions from many feed streams.

Case Study

In this study, five wash buffers were evaluated. A buffer containing 0.5 M MES offered high purity and yield, and a more economical solution of 1 M NaCl, 50 mM Na-phosphate also provided excellent purity and recovery.

A prominent target-related low molecular weight impurity was removed in bind/elute operation utilizing mixed-mode chromatography (MMC) following the affinity chromatography step.

Isolation of rHSA From Rice Extract

Transgenic rice expresses recombinant HSA at high levels and provides an economical source of rHSA. This study demonstrates the high binding capacity of rHSA to AVIPure Albumin resin and the compatibility of the resin with a range of wash and elution buffers. Hydrolyzed rHSA fragments are easily removed with a multi-mode polishing step.

Table 1. Operating Conditions for AVIPure Albumin Affinity Resin

Phase	Buffer	CV
Sanitization	0.5 M NaOH	3
Equilibration	150 mM NaCl, 50 mM citrate, pH 4.5	8
Load	Rice extract with 2 g/L rHSA, pH 4.5	28
Chase	150 mM NaCl, 50 mM citrate, pH 4.5	5
Wash	1, see Table 3	5
Elution	0.1 M citrate pH 3.0	10
CIP 1	0.1 M phosphoric acid	2
CIP 2	0.1 M NaOH	5
Re-equilibration	150 mM NaCl, 50 mM citrate, pH 4.5	8

Table 2. Column Specifications

Column Volume	Type of Column	Column Dimensions	Residence Time
1 mL	OPUS® MiniChrom® prepacked	5 x 0.5 cm	4 min
0.4 mL	Lab-packed	5 x 0.3 cm	4 min

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Figure 1. Purification of HSA From Rice Extract

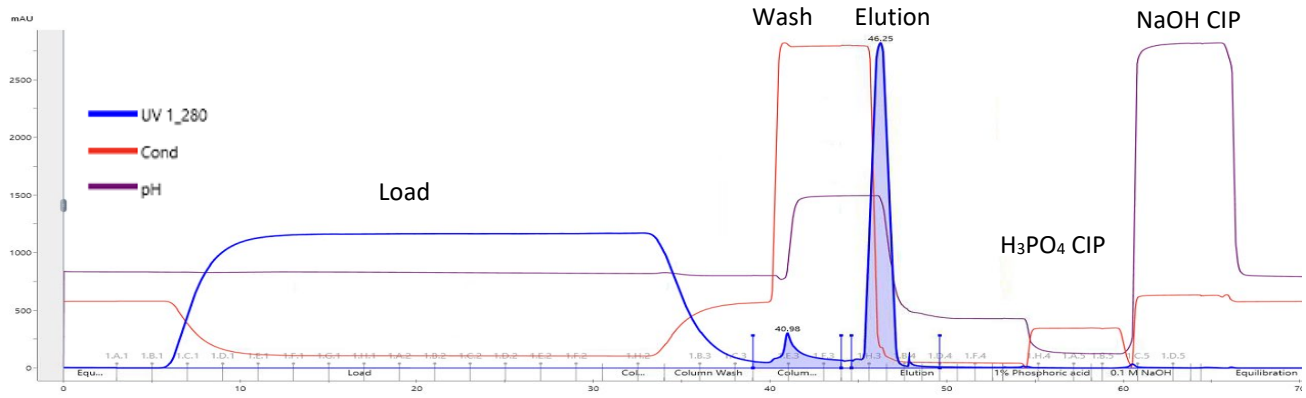
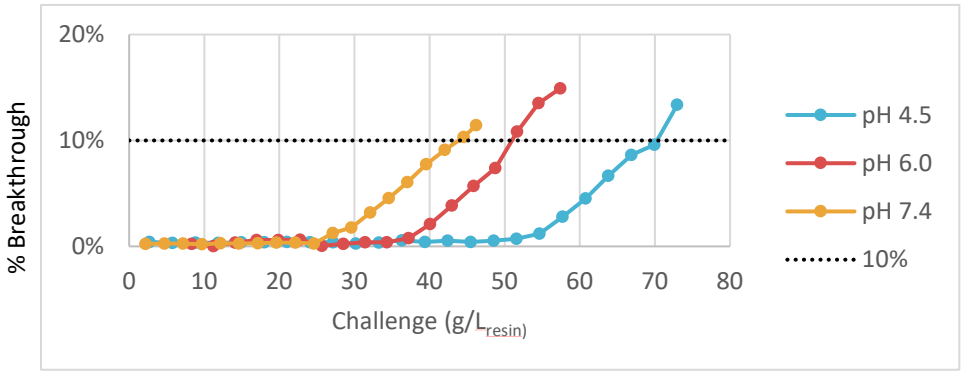


Figure 2. Dynamic Binding Capacity Study Using Rice Extract at Different pH Values



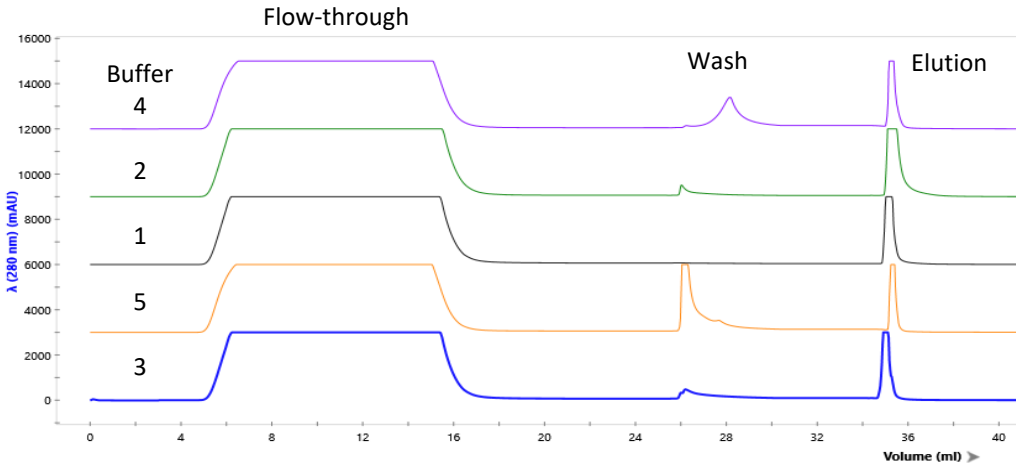
Load pH	DBC_{10}
4.5	70
6.0	50
7.4	45

Table 3. HP-SEC Analysis of Yield and Purity of Elution Pools under Various Wash Conditions

Buffer #	Wash Condition	% Yield	rHSA, %	HMW, %	LMW, %
1	150 mM NaCl, 50 mM citrate, pH 4.5	83%	76.5	3.7	19.8
2	0.5 M MES, 25 mM tris, pH 7.4	81%	77.9	3.9	18.1
3	1 M NaCl, 50 mM Na-phosphate, pH 7.4	75%	74.1	4.3	21.6
4	0.1 M NaSCN, 25 mM tris, pH 7.4	53%	65.6	6.2	28.2
5	1.5 M NaSCN, 25 mM tris, pH 7.4	20%	58.3	11.5	30.2

High
Low

Figure 3. Chromatographic Comparison of Wash Buffers (Table 3)



Results

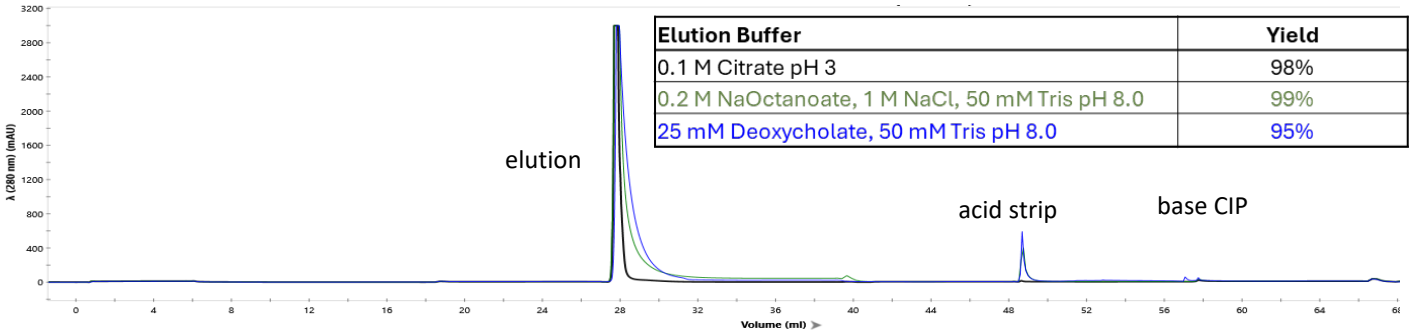
Figure 1 presents an example of rHSA affinity capture from rice extract. The column was loaded with 56 g rHSA/L_{res} using rice extract with 2 g/L rHSA. The wash buffer (1 M NaCl, 50 mM Na-phosphate, pH 7.4) removed weakly bound impurities, and the elution buffer (0.1 M citrate pH 3) released a sharp rHSA peak. Very little material eluted in the 0.1 M NaOH strip peak, indicating minimal/no resin fouling occurred during the experiment.

In a separate experiment, a column was loaded with extract to breakthrough at pH 4.5, 6, and 7.4. The 10% breakthrough point was determined by SDS-PAGE and HP-SEC. A capacity of 70 g rHSA/L_f was observed at pH 4.5 (Figure 2). Such a high capacity can greatly improve the process economics, enabling the use of smaller columns and less buffer, and can result in elution pools with higher titers and lower volumes.

Five wash buffers were evaluated for purity and yield (Table 3, Figure 3). The size of the wash peak is inversely correlated with the yield of rHSA. The purities of the neutralized eluates were determined by HP-SEC. The peak areas corresponding to rHSA, high molecular weight (HMW) contaminants, and low molecular weight (LMW) contaminants (Table 3) are presented sorted by yield. Wash buffers 1 (150 mM NaCl, 50 mM citrate, pH 4.5) and 2 (0.5 M MES, 25 mM tris, pH 7.4) achieved yields >80%, as well as the highest purities. Wash buffer 3 (1 M NaCl, 50 mM Na-phosphate, pH 7.4) had slightly lower yield and purity relative to Buffers 1 and 2. Buffers containing sodium thiocyanate washed off significant amounts of rHSA, resulting in lower yields (Table 3, Figure 3, buffers 4 and 5). Overall, buffers 1-3 offered excellent yield and purity, as judged by HP-SEC. HP-SEC alone was used to measure purity because no host-cell protein ELISA kit for rice extracts was available at the time of the experiment.

To accommodate both a variety of process needs and albumin fusion proteins that may not be stable at pH 3, two non-acidic elution buffers were evaluated (Figure 4). Columns were loaded with purified rice rHSA. All elution buffers delivered yields of 95% or greater, however relative to the pH 3 elution, there is noticeable peak tailing. This demonstrates that AVIPure Albumin offers flexible elution formulations that can be tailored for a given target and process.

Figure 4. Low and Neutral pH Elution Scouting



Frequently, rHSA feeds such as this rice extract contain significant levels of LMW product-related impurities, likely resulting from proteolysis of the target. This problem is reflected in the relatively large LMW peak areas post AVIPure capture (Table 3). Because these LMW contaminants are fragments of albumin, it is impossible for the AVIPure affinity ligand to differentiate these species from the target. Fortunately, these LMW impurities are easily removed in an efficient bind/elute step using multi-modal chromatography (MMC) resin. Table 4 outlines the chromatography parameters for the polishing step, and Figure 5 presents a sample chromatogram. In this example, the AVIPure eluate was adjusted to pH 4.5 and applied to a 1 mL Cytiva Capto™ MMC column at a challenge of 30 grams rHSA/L_{res} . The column was washed with buffers containing sodium chloride and ethanol (Table 4), and full-length rHSA was eluted in a neutral pH buffer. The LMW product-related impurities remained tightly bound, eluting during the CIP phase in 0.1 M NaOH. The overall MMC purification is summarized with SDS-PAGE and HP-SEC data in Figure 6. The LMW rHSA fragments are present in the AVIPure eluate, but not the MMC eluate. The fragments are recovered in the MMC strip. HP-SEC analysis reveals complete removal of the LMW peak that corresponds to fragmented rHSA. For comparison, rice rHSA from Sigma was analyzed by HP-SEC, and this material contained slightly more LMW fragments than the AVIPure captured, MMC-polished rHSA.

Table 4. Capto MMC Polishing Chromatography Protocol, 1 mL CV, 2 min Residence Time

Phase	Buffer	CV
Equilibration	25 mM citrate, 250 mM NaCl, pH 4.5	5
Load	AVIPure Eluate with 7.5 g/L rHSA, pH 4.5	4
Chase	25 mM citrate, 250 mM NaCl, pH 4.5	5
Wash 1	10% EtOH, 25 mM citrate, 250 mM NaCl, pH 4.5	5
Wash 2	15% EtOH, 25 mM citrate, 1 M NaCl, pH 4.5	5
Elution	25 mM Na-phosphate, 400 mM NaCl, pH 7.2	5
CIP	0.1 M NaOH	5

Figure 5. Capto MMC Chromatogram

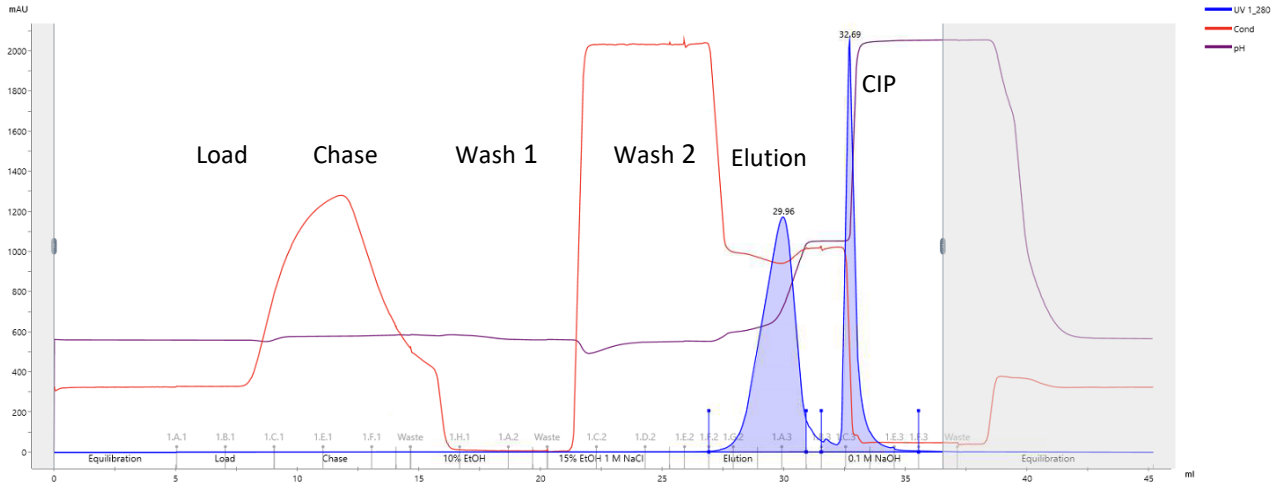
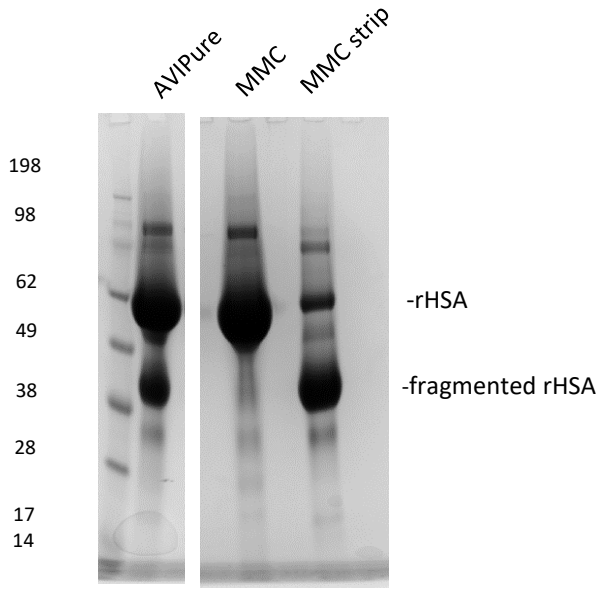
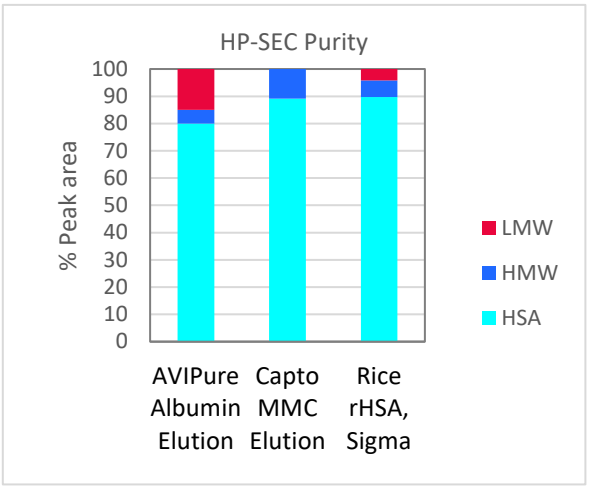


Figure 6. Purity After AVIPure Capture and Multi-Mode Chromatography Polishing to Remove Fragmented rHSA





Summary

AVIPure Albumin offers an efficient and cost-effective solution for purification of recombinant albumin from rice extracts. Extremely high capacities, economical wash and elution buffers, and high caustic stability give best-in-class process economics. AVIPure binds 70 g rHSA/L_{res} at pH 4.5 (Figure 2), is compatible with common wash buffers (Figure 3, Table 3), and offers both acidic and neutral elution options (Figure 4). Fragments of rHSA are a common impurity in many feedstocks, including rice extracts. Because they contain the same surface epitopes as intact rHSA, the AVIPure Albumin affinity ligand cannot discriminate between fragmented and full-length. A second purification step is required, and the MMC protocol outlined (Table 4, Figure 5, Figure 6) is an efficient and robust method for removing the LMW rHSA target-related impurities.

AVIPure Albumin has robust stability in sodium hydroxide, with no decrease in yield, capacity, or purity with up to 45 hours in 0.5 M NaOH. In addition to high capacity for rHSA, this resin can bind large albumin fusion proteins at 30 g/L_{res} capacity (see AVIPure Albumin Affinity Resin User Guide) and can be used with a range of common wash solutions. AVIPure Albumin is an excellent choice for the most challenging feed streams and an economical solution due to the high binding capacity and the tolerance of rigorous,

high-caustic CIP cycles for extensive reuse. Additionally, the more times a resin is cleaned and re-used, the lower the environmental impact of the bioprocessing step.

Ordering Information

Contact your account manager for sales, or, in some regions, you may purchase online at https://store.repligen.com/
You can also contact customer service at the email addresses for the regions listed below:

US: customerserviceUS@repligen.com
EU: customerserviceEU@repligen.com
China: customerserviceCN@repligen.com

Description	AVIPure Albumin
Affinity resin, 10 mL	100HSA-10
Affinity resin, 25 mL	100HSA-25
Affinity resin, 100 mL	100HSA-100
Affinity resin, 500 mL	100HSA-500
Affinity resin, 1 L	100HSA-1L
Affinity resin, 5 L	100HSA-5L
600 µL RoboColumn® - Strip of eight columns, 0.5 x 3 cm	23052108R-30
1 mL Pre-packed MiniChrom® Column, 0.5 x 5 cm	23052106
5 mL Pre-packed MiniChrom® Column, 0.8 x 10 cm	23051004-100

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