AVIPure® Albumin Affinity Resins

User Guide

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Abbreviations

As	Asymmetry
Bar	Equal to 100,000 Pascal
C	Celsius
CCCF	Clarified cell culture fluids
CF	Compression factor
cm	Centimeter
CV	Column volumes
DNA	Deoxyribonucleic acid
HCDNA	Host cell DNA
HCP	Host cell protein
HETP	Height equivalent to a theoretical plate
M	Molar
MES	(2-(N-morpholino)ethanesulfonic acid)
MgCl2	Magnesium chloride
mL	Milliliter
mm	Millimeter
mM	Millimolar
MPa	Megapascal
NaCl	Sodium chloride
NaOH	Sodium hydroxide
pH	A measure of how acidic/basic a solution is
psi	Pounds per square inch
TMAC	Tetramethyl ammonium chloride
µm	Micrometer or Micron, a metric unit of measure for length equal to 0.001 mm

1. AVIPure Albumin Affinity Resin

AVIPure® Albumin Affinity Resin is an alkali-tolerant resin for simple, one-step purification of human albumin proteins directly from clarified cell culture fluids (CCCF).

Table 1. Performance Characteristics of AVIPure Albumin Affinity Resin

Category	Description
Base matrix	Cross-linked agarose, spherical
Particle size (d_{50v})	70 µm
Ligand	Alkali-tolerant recombinant protein (animal free)
Coupling chemistry	Epoxide
Binding capacity	>30 g/L_{resin} recombinant human albumin at pH 7.4 and 4 minutes residence time >60 g/L_{resin} recombinant human albumin at pH 5 and 4 minutes residence time
Buffer compatibility	Stable to all commonly used aqueous buffers, including 8 M urea, 6 M guanidine hydrochloride, ethylene glycol, and detergents
Solvent compatibility	Water, alcohol (0 – 20% v/v), acetonitrile, 1 – 2 M acetic acid, other common organic solvents
pH stability	1 – 13
Cleaning-in-place stability	0.1 – 0.5 M NaOH
Pressure/flow[a]	3 bar at >300 cm/hr
Maximum pressure (ΔP)[a]	40 psi
Temperature stability	2 – 40 °C
Delivery conditions	2% benzyl alcohol (slurry), or 20% ethanol (pre-packed columns)
Storage	2 – 8 °C, 2% benzyl alcohol or 20% ethanol; do not freeze

[a] In a 2.6 x 20 cm column pressure packed at 4 bar.

Key performance attributes of AVIPure Albumin Affinity Resin include:

- Binds human albumin and human albumin proteins with high capacity in typical cell culture conditions
- Clean with 0.5 M NaOH
- Reduce residual host cell protein (HCP) and host cell DNA (HCDNA)
- Ability to elute at neutral pH
- Use with standard bioprocess columns and relevant process flowrates

2. Process Development Recommendations

Optimal conditions for purification of albumin using AVIPure Albumin Affinity Resin must be determined empirically for each source. Some general process development recommendations for identification of optimal process conditions are provided below and summarized in Table 2. For the most up to date application notes, please refer to https://www.repligen.com/resources.

Table 2. Recommended Starting Purification Protocol for AVIPure Albumin Affinity Resin

Step	Column Volumes	Residence Time	Suggested Buffer
Sanitization	3 – 5	4 – 6	0.5 M NaOH
Equilibration	8	4	150 mM Sodium chloride, 20 mM Sodium phosphate, pH 7.4 Generally, equilibration can be matched to lysis buffer
Load	Concentration dependent	4	Buffers must have conductivity ≥10 mS/cm to allow binding *Note that lower load pH results in higher capacity (>60 g/L_{resin})*
Wash 1	5	4	Equilibration buffer
Wash 2 (if needed)	5	4	1 M NaCl, 20 mM Sodium phosphate, pH 7.4
Wash 3	2	4	Equilibration buffer
Elution	5	4	50 mM citrate, pH 3 (collect peak based on UV280 and neutralize elution pool with 1 M Tris, pH 9) See section 2.3 for higher pH elution conditions
Strip	2	4	Process specific (e.g., pH <2.0)
CIP[a]	5 or 1	6 or 30	0.5 M NaOH
Re-equilibration	8	4	Equilibration buffer

[a]Total contact time for CIP should be 30 minutes.

The agarose base bead enables use in typical bioprocess column diameters and bed heights (5 – 20 cm).

2.1 Equilibration and Binding Conditions

Binding of albumin to AVIPure Albumin Affinity Resin has been demonstrated with buffers at near neutral pH (5 – 9) and over a wide range of ionic strength (100 – 500 mM NaCl). Lower load pH leads to an increase in capacity for human albumin of greater than 60 g/L_{resin}. Salt concentrations greater than 500 mM NaCl have not been tested. Most conventional buffers (e.g., phosphate, citrate, acetate, Tris) may be used during equilibration and loading.

2.2 Wash Conditions

Optimized wash conditions ensure high purity protein is eluted from the resin. After loading the feed stock, washing unbound material with five column volumes (CV) of equilibration buffer is recommended. An additional intermediate wash step can further increase final purity. Screening diverse wash buffers (pH 4 – 9) can help reduce host cell proteins (HCP). Examples of wash additives that can be used at neutral pH that have proven effective for increasing purity of human albumin are:

- 0.05 – 0.1 M Sodium thiocyanate
- 0.25 – 0.5 M MES
- 1 M Magnesium chloride; Note that magnesium is poorly soluble with phosphate. If using magnesium in the Wash 2 buffer, consider using a solution that is not buffered with phosphate for Wash 1 and Wash 3.

2.3 Elution Conditions

Albumin can be eluted from the affinity resin with low pH buffers (e.g., pH 3.0, see Table 2 above). Effective elution at pH 3.5 can be achieved with the addition of 10% (v/v) hexanediol. If higher pH elution conditions are required, guidance is provided below for the range pH 8 – 9. In all instances, immediate pH neutralization of the elution buffer can help maintain product integrity.

If elution at higher pH is desired, 200 mM sodium octanoate, 1 M NaCl, 50 mM HEPES at pH 8.0 can be used, though an intermediate step must be included to flush the sodium octanoate from the system prior to the strip step to prevent precipitation. Alternatively, 20% (w/v) hexanediol, 1 M NaCl, 50 mM HEPES at pH 8.0 is also effective. Addition of 0.5 M proline to the hexanediol buffer has been shown to reduce elution peak tailing and reduce the concentrations required for both hexanediol and sodium chloride. For example, 15% (w/v) hexanediol, 0.5 M proline, 50 mM glycine, pH 9.0 was shown to effectively elute target protein within 2 – 3 column volumes.

Combinations of additives can act synergistically for elution and should be evaluated for improved elution yield. Step elution can achieve high product concentrations; product typically elutes in two to three column volumes.

2.4 Cleaning-In-Place and Sanitization Conditions

The alkaline tolerance of AVIPure Albumin Affinity Resin supports the use of 0.5 M NaOH for cleaning in place (CIP). A CIP process of 0.5 M NaOH exposure for 30 minutes per cycle can help maintain consistent chromatographic performance for 30 cycles of CCCF challenge. A robust CIP process can help maintain the consistency of key process parameters across multiple cycles, which include flow properties, residual HCP and DNA levels, and binding capacity. Upflow based CIP may be used. The following CIP protocol represents a starting point for the AVIPure Albumin Affinity Resin.

1. Wash the column with 5 column volumes of equilibration buffer
2. Apply 5 column volumes of 0.5 M NaOH at a 6-minute residence time or perform a static hold for a total contact time of 30 minutes. NaOH concentration and contact time exposure should be empirically determined for each construct and process.
3. Re-equilibrate the column with ≥5 column volumes of equilibration buffer.

3. Storage

Bulk AVIPure Albumin Affinity Resin is stored in 2% benzyl alcohol, and packed columns in 20% ethanol. Keep unused resin in its original container and store at 2 – 8 °C. Do not freeze. After sanitization and neutralization, store packed columns at room temperature (short term) or at 2 – 8 °C (long term) with an appropriate bacteriostatic agent such as 20% ethanol or 2% benzyl alcohol.

4. Column Packing

AVIPure Albumin Affinity Resin can be ordered in OPUS® Pre-packed Columns or as a loose slurry for self-packing.

AVIPure Albumin Affinity Resin is based on a 70 µm highly cross-linked rigid agarose base matrix developed for bioprocess applications. Pack in bioprocess column sizes with standard procedures developed for similar chromatography resins.

Pack laboratory-scale and small-scale production columns according to the following instructions: The AVIPure Albumin Affinity Resin storage solution should be exchanged with purified water or 100 mM NaCl before packing into a column. The packing solution should be filtered and degassed prior to use.

Exchange of the storage solution can be done by either: (1) repeatedly settling the resin, decanting the buffer and re-mixing the resin in the packing buffer, or (2) pouring the resin slurry into the column, draining off the storage solution, and replacing it with the packing buffer. In either case, equilibrate the resin slurry temperature in the packing location prior to the buffer exchange procedure.

The recommended compression factor, the ratio of the settled bed height prior to column packing to the final bed height, is 1.2. If flow packing with buffer recycling, the minimum volume of packing buffer required is 3–4 times the packed bed volume.

If packing a column to more than 50% of the column hardware length, use an extension reservoir.

1. Ensure the storage solution has been fully removed and the resin is in packing buffer at a slurry concentration between 45 and 60%. Magnetic stir bars are not recommended for slurry mixing due to potential damage to the resin from grinding against the container surface.
2. Calculate the slurry volume by dividing the column volume by the slurry concentration and multiplying the obtained ratio by the compression factor (CF).
3. Clean column hardware and frits.
4. Secure the column in a vertical and plumb position.
5. Wet the surface of the bottom frit with a small volume of packing solution.
6. Mix the packing slurry fully. Gently pour the slurry down the side of the column using care to ensure that air is not trapped in the slurry as the column is filled.

7. Fill the column with packing buffer completely. Remove air from the inlet flow adapter by flowing packing buffer through the tubing and frit. Stop the flow and attach the inlet fitting to the column ensuring that no air is trapped between the inlet frit and the slurry (hint: insert the top adapter at a 45° angle).
8. Confirm the expected compression factor when the resin has settled to the target bed heights.
9. For the Omnifit™ 10/100 column, follow steps 10a – 10e. For HiScale™ 16 or 26 columns, follow steps 11a – 11e.
10. Omnifit 10/100 Columns.
    a. Open the column outlet and start packing buffer flow at 200 cm/h to remove air from the flow adaptor. Stop the flow and bring the top adaptor to approximately 1 mm above the bed formation. Restart the flow and connect the bottom tubing to the system.
    b. Continue flowing and bring the adapter down to the target bed height. No further compression is needed.
    c. Condition the packed column at 200 cm/h by flowing 3 column volumes of packing buffer upflow, followed by 3 column volumes downflow.
    d. Repeat step b three times (Note: check the pressure; for 5 cm bed height usually it is less than 3 bar = 0.3 MPa; if a gap has formed, lower the adapter and repeat the steps b – c).
    e. The column is now ready to be tested.
2. HiScale 16 OR 26 Columns
    a. Open the column outlet and start packing buffer flow at 300 cm/h.
    b. Continue compressing the bed by flow for approximately 20 minutes.
    c. Stop the flow and disconnect the tubing from the top of the column.
    d. Manually compress the bed by adjusting the adaptor until the target bed height is reached.
    e. The column is now ready to be tested.

5. Column Integrity Testing

Test for mechanically correct packing by the application of either an acetone spike or high salt spike and recording the resultant peak. This test can also be used between runs to evaluate changes in bed integrity.

Evaluate the column packing efficiency by using a 2% CV plug injection of 1 – 2% acetone in packing buffer. This test should be conducted at a low linear velocity, typically around 30 – 60 cm/hr. Calculate the number of theoretical plates (N), the reduced plate height (h) from the plate height (HETP) and the peak asymmetry (As) by standard procedures described by the following equations:

$$N = 5.54 \times \left(\frac{Vr}{W_h}\right)^2$$

$$HETP = \frac{L}{N}$$

$$h = \frac{HETP}{d_{50v}}$$

$$As = \frac{b}{a}$$

where V_r is volume eluted from the start of the sample application to the peak maximum, W_h is the width of the recorded peak at half of the peak height (V_r and W_h have the same units, e.g., CV, time, volume), L is bed height (cm), d_{50v} is mean particle size (cm; for AVIPure Albumin, d_{50v} = 0.007 cm), b and a are widths of descending and ascending parts of the peak measured at 10% of the peak height, respectively.

For a well packed AVIPure Albumin Column, expected quality limits include:

- Asymmetry (As): 0.8 – 2
- Reduced height equivalent of a theoretical plate (h): <4

6. 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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