Albumin Binding Domain Affinity Resin

User Guide

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Abbreviations

ABD	Albumin binding domain
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
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
µm	Micrometer or Micron, a metric unit of measure for length equal to 0.001 mm

1. Albumin Binding Domain (ABD) Affinity Resin

Albumin Binding Domain (ABD) Affinity Resin is an alkali-tolerant resin for simple, one-step purification of ABD-fused molecules directly from clarified cell culture fluids (CCCF).

Table 1. Performance Characteristics of ABD Affinity Resin

Category	Description
Base matrix	Cross-linked agarose, spherical
Particle size (d_{50v})	70 µm
Ligand	Recombinant protein (animal free)
Coupling chemistry	Epoxide
Binding capacity	≥18 g/L_{resin} ABD-fused protein at 5 minutes residence time
Buffer compatibility	Stable to aqueous buffers as recommended in Table 2. Other buffers have not been tested.
Solvent compatibility	Water, ethanol (0 – 20% v/v), 100 mM acetic acid. Other solvents have not been tested.
Cleaning-in-place stability	0.1 M NaOH
Pressure/flow[a]	3 bar at >300 cm/hr
Maximum pressure (ΔP)[a]	40 psi
Delivery conditions	20% ethanol (pre-packed columns)
Storage	2 – 8°C, 18 – 20% ethanol; do not freeze

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

Key performance attributes of ABD Affinity Resin include:

- Binds ABD-fused molecules with high capacity in typical cell culture conditions
- Clean with 0.1 M NaOH
- Reduce residual host cell protein (HCP) and host cell DNA (HCDNA)
- Use with standard bioprocess columns and relevant process flowrates

2. Process Development Recommendations

Optimal conditions for purification of ABD-fused molecules using ABD 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 ABD Affinity Resin

Step	Column Volumes	Residence Time	Suggested Buffer
Equilibration	5	2	200 mM Sodium chloride, 50 mM Sodium phosphate, pH 7.0
Load	Concentration dependent	5	pH adjusted to 6.7 – 7.3 with 0.5 M Na_2HPO_4Conductivity adjusted to ~25 mS/cm with 2 M NaCl
Wash 1	10	5	Equilibration buffer
Elution	6	5	100mM Acetic Acid, pH 3 • Collect peak based on UV280 • Neutralize elution pool with 1 M Tris, pH 8.5
Neutralization	3	2	Equilibration buffer
CIP[a]	4	4	0.1 M NaOH
Re-equilibration	3	2	Equilibration buffer
Storage	3	6 – 8[b]	18 – 20% ethanol

[a]Total contact time for CIP should be at least 15 minutes.
[b]Residence time depends on bed height and delta column pressure.

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 ABD-fused molecules to ABD Affinity Resin has been demonstrated with buffers at near neutral pH (6.7 – 7.3) and conductivity up to 30 mS/cm.

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 ten column volumes (CV) of equilibration buffer is recommended.

2.3 Elution Conditions

ABD-fused molecules can be eluted from the affinity resin with 100 mM acetic acid at pH 3 (Table 2). Collect the elution peak based on UV280. If the target protein is sensitive to low pH, neutralize the elution pool with 1 M Tris, pH 8.5.

2.4 Cleaning-In-Place and Sanitization Conditions

The alkaline tolerance of ABD Affinity Resin supports the use of 0.1 M NaOH for routine cleaning in place (CIP). A CIP process of 0.1 M NaOH exposure for at least 15 minutes per cycle can help maintain consistent chromatographic performance for 50 cycles of CCCF challenge. Occasional sanitization with 0.5 M NaOH (e.g., every 5th cycle, or prior to storage) may also be employed.

A robust CIP process can help maintain the consistency of key process parameters across multiple cycles, including 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 ABD Affinity Resin.

1. Wash the column with 3 column volumes of equilibration buffer.
2. Apply 3 column volumes of 0.1 M NaOH for a total contact time of 15 minutes or perform a static hold with 0.1 M NaOH for a total contact time of at least 15 minutes. NaOH concentration and contact time exposure should be empirically determined for each construct and process.
3. Re-equilibrate the column with ≥3 column volumes of equilibration buffer.

3. Storage

Bulk ABD Affinity Resin is stored in 18 – 20% ethanol, 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 18 – 20% ethanol.

4. Column Packing

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

ABD 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.

4.1 Column Packing Instructions

The resin is supplied in 18% – 20% ethanol. In preparation for column packing, exchange the shipping solution with 0.1 M sodium chloride. Alternatively, phosphate buffered saline (PBS) or water can be used as the packing buffer. The resin may be packed using flow pack or axial compression methods.

4.1.1 Flow Packing

1. Decant storage solution and resuspend resin in the desired packing buffer.
2. Attach bottom flow adaptor to column body.
3. Transfer the resin slurry into the column. Take into account a target compression factor (CF) of 1.2 in order to achieve the desired final column volume (CF of 1.1 – 1.3, depending on the packing pressure).
4. Close the outlet port of the column and connect the top flow adapter to the tubing of the three-way valve labeled To Column.
5. Set the three-way valve to the *pump to purge* position and prime the flow path.
6. Set the three-way valve to the *column to purge* position. Lower the adapter into the column and allow liquid to vent through the three-way valve purge line until air is purged.
7. Lock flow adapter in place. Set the three-way valve to the *pump to column* position. Open the bottom port of the column and flow at 100 cm/hr until bed has formed. Stop the pump. Close the bottom column port.
8. Set the three-way valve to the *column to purge* position. Manually lower the flow adaptor until it reaches 0.5 – 1 cm above the settled bed. Liquid should purge through the top of the column via the three-way valve.
9. Set the three-way valve to the *pump to column* position. Open the bottom column port and flow buffer up to a pressure of 2.5 – 3 bar ΔP and mark the top of the resin bed once stabilized. Maintain flow for a minimum of 3 CV. Stop the pump. Close the bottom column port.
10. Set the three-way valve to the *column to purge* position and manually lower the flow adapter to the bed height marked in the previous step.
11. Set the three-way valve to *pump to column* position. Open the bottom column port. Flow buffer for 3 CV at a flow rate that creates 2.5 – 3 bar to condition the column. If a gap forms between the flow adaptor and the bed, lower the adapter and repeat the previous step. Do not run at the packing pressure again or the column will continue packing down.
12. Evaluate column performance using HETP and asymmetry.
    a. HETP: >2,000 N/m
    b. Peak asymmetry: 0.8 – 1.8

Note: *If HETP is <2500 N/m and asymmetry >1.8, increase compression factor by lowering the flow adapter in 0.02 CF increments and retest. If asymmetry <0.8, reduce compression in 0.02 CF increments and retest.*

4.1.2 Axial Compression

1. Decant storage solution and resuspend resin in the desired packing buffer.
2. Attach bottom flow adaptor to column body.
3. Transfer the resin slurry into the column. Take into account a target compression factor (CF) of 1.2 in order to achieve the desired final column volume (CF of 1.1 – 1.3, depending on the packing pressure).

4. Close the outlet port of the column and connect the top flow adapter to the tubing of the three-way valve labeled To Column.
5. Set three-way valve to the *pump to purge* position and prime the flow path. Close the outlet port of the column and connect the flow adapter to the top of the column.
6. Set the three-way valve to the *column to purge* position. Lower the adapter into the column and allow liquid to vent through the top port until air is purged.
7. Lock flow adapter in place. Set the three-way valve to the *pump to column* position. Open the bottom port of the column and flow at 100 cm/hr until bed has formed. Stop the pump.
8. With the pump stopped, keep the three-way valve in the *pump to column* position and the bottom outlet port open, lower the flow adaptor at a rate of 150 cm/hr until the target compression factor is achieved (liquid will flow out of the bottom port of the column).
9. Flow condition the column with an additional 3 CV packing buffer at a flow rate that achieves 2.5 – 3 bar. If a gap forms between the flow distributor and the bed, lower the adapter and repeat flow condition step. Do not run at the packing pressure again or the column will continue packing down.
10. Evaluate column performance using HETP and asymmetry.
    a. HETP: >2,000 N/m
    b. Peak asymmetry: 0.8 – 1.8

Note: *If HETP is <2500 N/m and asymmetry >1.8, increase compression factor by lowering the flow adapter in 0.02 CF increments and retest. If asymmetry <0.8, reduce compression in 0.02 increments and retest.*

5. Column Integrity Testing

Column qualification is typically determined by testing HETP (height equivalent to a theoretical plate) and As (peak asymmetry).

IMPORTANT: For best results, avoid sample dilution by applying the sample as close to the column inlet as possible, and placing the conductivity meter as close to the column outlet as possible.

Table 33. Recommended Column Efficiency Testing Parameters

Condition	Recommendation
Detection	Conductivity
Effluent solution	0.1 M NaCl
Sample volume	1% of the column volume
Sample concentration	1 M NaCl
Flow rate	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 ABD, 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 ABD Column, expected quality limits include:

- Asymmetry (As): 0.8 – 1.8
- HETP: > 2500 N/m

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

Table 4. Part Numbers

Description	Part Number
ABD Affinity resin, 25 mL	ABD-AR-0025
ABD Affinity resin, 100 mL	ABD-AR-0100
ABD Affinity resin, 1 L	ABD-AR-1L
ABD Affinity resin, 5 L	ABD-AR-5L

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