

Certificate of Analysis

SoftLink™ Soft Release Avidin Resin:

Part No.	Size
V201A	1ml
V201B	5ml

Description: SoftLink™ Soft Release Avidin Resin^(a) in approximately 20% ethanol.

Part No.	Bed Volume	Total Volume
V201A	1ml	2ml
V201B	5ml	10ml

SoftLink™ Soft Release Avidin Resin can be used for the isolation and purification of biotinylated molecules. SoftLink™ Resin is a rigid, methacrylate polymeric gel filtration matrix, functionalized with covalently bound, monomeric avidin. Monomeric avidin binds biotin with a $K_d = 10^{-7}$, allowing reversible binding of bound biotinylated proteins under mild elution conditions. Native, or tetrameric, avidin binds biotin with a very strong affinity ($K_d = 10^{-15}$) and requires strong denaturing conditions to elute bound material. Monomeric avidin allows the specificity of capture and the mild elution appropriate for the purification of sensitive biological materials.

No detectable leaching of avidin under biotin elution conditions occurs because monomeric avidin is covalently linked to the polymer. SoftLink™ Resin supports high flow rates (300cm/hr) and centrifugal forces ($1,500 \times g$) in batch operation. SoftLink™ Resin retains the ability to bind biotin following exposure to pH 2–13, low or high ionic strength (up to 2M NaCl), 6M guanidine, and 1% SDS. Up to 4mg of biotinylated protein has been purified per milliliter of SoftLink™ Resin.

Storage Conditions: Store at 4°C or room temperature. **Do Not Freeze.**

Quality Control Assays

Binding Capacity: Specifications require a minimum recovery of 30nmol of biotinylated cytochrome c bound per milliliter of resin. Cytochrome c is eluted in 10% acetic acid, and protein concentration is determined spectrophotometrically at 528nm using an extinction coefficient of $0.905\text{ml}\cdot\text{mg}^{-1}\cdot\text{cm}^{-1}$ for biotinylated cytochrome c.

Microbial Contamination: Minimum specifications require $<100\text{cfu/ml}$ of resin.

Particle Size: 40–90 microns, with $<10\%$ passing through a $45\mu\text{m}$ filter (1,000Å pore size).

Part# 9PIV201

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Signed by:

R. Wheeler, Quality Assurance

1. Purification of Biotinylated Proteins

A. Preadsorption of SoftLink™ Resin with Biotin

Perform at 4°C or room temperature.

1. Pour a column with appropriate bed volume.
2. Equilibrate the column in 0.1M NaPO₄ (pH 7.0).
3. Preadsorb nonreversible binding sites on fresh SoftLink™ Resin by washing with 5mM biotin in the phosphate equilibration buffer. Wash with a flow rate of 6ml of biotin/hour/ml of SoftLink™ Resin until two column volumes have passed through.
4. Stop the flow for 15 minutes to allow biotin binding.
5. Regenerate the column before attempting to purify biotinylated molecules.

Regeneration Conditions

Perform at 4°C or room temperature.

1. Wash the column with 8 column volumes of 10% acetic acid.
2. Wash the column with 8 column volumes of 100mM NaPO₄ (pH 7.0).
3. Monitor pH of eluate until it reaches pH 6.8. Stop the flow for a minimum of 30 minutes to allow the avidin to refold.
4. Equilibrate the column in a buffer compatible with the biotinylated molecule to be purified.

Suggested Equilibration Buffers: 50mM Tris-HCl (pH 8.0) or 50mM Tris-phosphate (pH 7.8) with or without one or more of the following additives: 50–200mM NaCl, 4mM dithiothreitol, 2mM EDTA, 10% glycerol or 0.1% Triton® X-100.

5. Store the column in 20% ethanol for up to six months without loss of binding capacity.

B. Purification of Biotinylated Proteins from Solution

Perform at 4°C or temperature suitable for the biotinylated protein to be purified.

1. Load the sample in equilibration buffer at a flow rate of 6ml of sample/hour/ml of column bed volume. This flow rate may be optimized for a specific application and can be increased without concerns over SoftLink™ Resin compression.
2. Wash the column with about 5 column volumes of equilibration buffer.
3. Elute the biotinylated protein from the column with 5mM biotin in equilibration buffer.
4. Assay each fraction for protein concentration or monitor absorbance at 280nm.

Recovery Notes: After a biotinylation reaction, free biotin was dialyzed away from the reactants (IgG proteins) before the samples were bound to the SoftLink™ Resin. Ninety percent of the biotinylated antibody proteins were recovered in the elution step with biotin. The remaining 10% of the biotinylated IgGs were recovered in the acetic acid regeneration step.

Caution: Do not expose the SoftLink™ Resin to sodium hydroxide concentrations greater than 0.1N (pH 13). Exposure will significantly reduce binding capacity.