INSTRUCTIONS

# NeutrAvidin® UltraLink® Resin

## 53150 53151

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| Number | Description |
|---|---|
| **53150** | **NeutrAvidin UltraLink Resin,** 5mL settled resin, supplied as 50% slurry (10mL total volume) in 0.02% sodium azide<br>Binding Capacity: 12-20µg biotin/mL of settled resin |
| **53151** | **NeutrAvidin Plus UltraLink Resin,** 5mL settled resin, supplied as 50% slurry (10mL total volume) in 0.02% sodium azide<br>Binding Capacity: ≥ 30µg biotin/mL of settled resin |

**Storage:** Upon receipt store at 4°C. Product is shipped at ambient temperature.

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## Introduction

The Thermo Scientific NeutrAvidin UltraLink Resin can be used to separate biotinylated products from non-biotinylated products and to affinity-purify proteins when used with biotinylated antibodies. NeutrAvidin Protein is a modified version of avidin with a molecular weight of approximately 60K. Unlike avidin, NeutrAvidin Protein is carbohydrate free and has a near-neutral isoelectric point (pI=6.3), resulting in minimal nonspecific binding. Also, NeutrAvidin Protein does not contain the RYD domain that is analogous to the universal recognition sequence for a variety of cell adhesion receptors, which eliminates the potential for binding to cells through these receptor molecules.

NeutrAvidin Protein immobilized onto UltraLink Resin provides an affinity support that is leak resistant and pH stable. The UltraLink Resin is an azlactone-activated support that is hydrophilic, charge-free, high capacity, highly crosslinked, rigid, copolymeric and porous. This support is especially useful for medium pressure methods when using large sample volumes requiring fast-flow techniques (FPLC) and large-scale applications. Agarose supports are useful for gravity-flow procedures; however, the more rigid UltraLink Resin is used if flow rates require pressures greater than 25psi. More specific information regarding this support is detailed in the Additional Information Section.


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## Important Product Information

- The flow rate of liquid through the column will be slow if gravity flow is used. For best results, attach the column to a peristaltic pump by placing flexible tubing over the bottom tip of the column. The column flow rate can then be accelerated and easily controlled.

  **Note:** For information and calculations for determining linear flow rate see the Additional Information Section.

- To elute biotinylated molecules from the NeutrAvidin Protein use 8M guanidine•HCl, pH 1.5 (Product No. 24115) or boil the resin with SDS-PAGE sample buffer. These harsh elution conditions may cause leaching of NeutrAvidin Protein Subunits into the sample.

  **Note:** These elution conditions may irreversibly damage the protein of interest. If non-denaturing elution conditions are required, use Monomeric Avidin (Product No. 20228), which allows mild elution of biotinylated molecules without contamination from subunits and without reducing the resin's binding capacity. Alternatively, biotinylate the protein using NHS-Iminobiotin (Product No. 21117), which binds to NeutrAvidin Protein at pH 9.5 and dissociates at pH 4. Alternatively, use a thiol-cleavable biotinylation reagent such as NHS-SS-Biotin (Product No. 21331).

- The protocols included in this instruction booklet are examples of applications for this product. Specific applications and systems will require optimization.

## Procedure for Separating Biotinylated Products

### A. Materials Required

- Biotinylated sample
- Binding Buffer: Phosphate-buffered Saline (e.g., 0.1M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372).
- Elution Buffer: 8M guanidine•HCl, pH 1.5 (Product No. 24115)
- Disposable column such as the Disposable Polypropylene Columns for 1.0-5.0mL resin-bed volumes (Product No. 29922) or the Disposable Column Trial Pack (Product No. 29925) that contains two each of three column sizes (i.e., 0.5-2.0mL, 1.0-5.0mL and 2.0-10.0mL resin-bed volumes). Alternatively, use a medium-pressure chromatography column.

  **Note:** For spin-column formats, use Mini-Spin Columns and Accessories (Product No. 69705).

### B. Procedure

1. Equilibrate the resin and reagents to room temperature.
2. Carefully pack a column with the desired amount of resin.
3. Equilibrate column with five resin-bed volumes of Binding Buffer.
4. Apply the biotinylated sample to the column and allow it to enter the resin bed. Stop the column flow and incubate for 30 minutes at room temperature.
5. Wash column with 10 resin-bed volumes of Binding Buffer.
6. Elute the bound biotinylated product from the column with five resin-bed volumes of Elution Buffer.
7. Exchange the solution of the eluted fractions by dialysis or gel filtration. Discard the used resin.

## Procedure for Affinity Purification

### A. Materials

- Biotinylated Antibody: Use approximately 3mg of biotinylated protein/ml of settled resin (2mL of the 50% slurry is equivalent to 1mL of settled resin). Prepare biotinylated antibody at 0.2-10mg/mL in Binding Buffer.
- Binding Buffer: Phosphate-buffered Saline (e.g., 0.1M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372).
- Elution Buffer: IgG Elution Buffer (Product No. 21004 or 21009) or glycine•HCl, pH 2.5-3.0
- Disposable column such as the Disposable Polypropylene Columns for 1.0-5.0mL resin-bed volumes (Product No. 29922) or the Disposable Column Trial Pack (Product No. 29925) that contains two each of three column sizes (i.e., 0.5-2.0mL, 1.0-5.0mL and 2.0-10.0mL resin-bed volumes). Alternatively, use a medium-pressure chromatography column.

**B. Procedure**

- The amount of antigen needed and the incubation time depends upon the antibody-antigen system used and must be determined empirically for each specific system.
- When using pre-packed columns, remove the top cap first and then remove bottom cap to prevent air bubbles from being drawn into the resin.

1. Equilibrate the resin and reagents to room temperature.
2. Carefully pack a column with the desired amount of resin. Equilibrate the column with five resin-bed volumes of Binding Buffer.
3. Apply the biotinylated antibody to the column and allow it to enter the resin bed. Stop the column flow and incubate for 30 minutes at room temperature or overnight at 4ºC.
4. Wash the column with 10 resin-bed volumes of Binding Buffer.
5. Apply the antigen solution to the column and allow it to enter the resin bed. Stop the column flow and incubate for 30 minutes at room temperature or overnight at 4ºC.
6. Wash the column with 10 resin-bed volumes of Binding Buffer.
7. Elute the antigen with 5-10 column volumes of Elution Buffer. Collect the eluate in 0.5-1mL fractions. If using IgG Elution Buffer or 0.1M glycine•HCl, pH 2.8, immediately neutralize the pH by adding 100µL of 1M Tris, pH 7.5 per 1mL of eluted sample. Monitor protein by measuring the absorbance of each fraction at 280nm.

   **Note:** If using Gentle Ag/Ab Elution Buffer, wash column with three column volumes of Tris-buffered saline before performing the elution. The Gentle Elution Buffer is not compatible with phosphate-based buffers.
8. Exchange the buffer of the eluted fractions by dialysis or gel filtration.

   **Note:** Wash the immobilized biotinylated-antibody column with 10 column volumes of binding buffer before using it to purify more antigen. To store column, add a final concentration of 0.02% sodium azide and store at 4°C.

## Procedure for Batch-format Immunoprecipitation

**Note:** Perform the following procedure in a microcentrifuge tube. Alternatively, using a Spin Cup Column (Product No. 69700) will improve separation of solutions from the resin.

**A. Additional Materials Required**

- Binding Buffer: Phosphate-buffered saline (e.g., 0.1 M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372). To reduce possible nonspecific binding add 0.1% SDS, 1% NP-40 or 0.5% sodium deoxycholate.
- Biotinylated Antibody: Use approximately 3mg of biotinylated protein/ml of settled resin (2mL of the 50% slurry is equivalent to 1mL of settled resin). Prepare 0.1-1.2mg of biotinylated antibody at 0.2-10mg/mL in Binding Buffer.
- Elution Buffer: IgG Elution Buffer (Product No. 21004), Gentle Ag/Ab Elution Buffer (Product No. 21027) or 0.1M glycine•HCl, pH 2.8
- Microcentrifuge tube(s) or Mini-Spin Columns and Accessories (Product No. 69705)
- SDS-PAGE sample buffer (optional): 2% SDS, 62.5mM Tris base, 10% glycerol, 2.5% 2-mercaptoethanol, pH 6.8

**B. Procedure**

- The amount of antigen needed and the incubation time are dependent upon the antibody-antigen system used and must be determined empirically for each specific system.
- To allow for proper mixing, make sure the total reaction volume does not completely fill the microcentrifuge tube.

1. Equilibrate the resin and reagents to room temperature.
2. To form the immune complex, add biotinylated antibody to the antigen sample or lysate and incubate for at least 30 minutes at room temperature or overnight at 4ºC.

3. Gently swirl the bottle of UltraLink Resin to obtain an even suspension. Pipette the appropriate amount of resin into a microcentrifuge tube or spin column. Centrifuge the tube for 1 minute at medium speed (i.e., 3000-5000 × *g*) and discard supernatant.
4. Wash resin twice by adding Binding Buffer and centrifuging for 1 minute at medium speed. Discard the supernatant.
5. Add the immune complex to the resin and incubate with mixing for 1 hour at room temperature or 4°C.
6. Wash the resin-bound complex with Binding Buffer and centrifuge for 1 minute at medium speed. The supernatant may be discarded or saved to evaluate binding. Repeat this wash procedure at least four times.
7. The sample may be boiled in SDS-PAGE sample buffer and electrophoresed for analysis. Alternatively, add Elution Buffer to the resin to recover the antigen. If using IgG Elution Buffer or 0.1M glycine•HCl, pH 2.8, remove liquid and immediately adjust the pH by adding a suitable concentrated buffer such as 1M Tris, pH 7.5 (100µL of this buffer to 1mL of the sample is sufficient).

## Additional Information

### A. Specific Physical Characteristics of the UltraLink Resin

The UltraLink Resin is an azlactone-activated support that is hydrophilic, charge-free, high capacity, highly crosslinked, rigid, copolymeric and porous (Table 1). The support characteristics are important considerations when using large sample volumes requiring fast-flow techniques and large-scale applications.

**Table 1. Characteristics of the Thermo Scientific UltraLink Resin.**

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| **Support pH Stability:** | 1-13 |
| **Average Particle Size:** | 50-80µm |
| **Exclusion Limit:** | > 2,000,000Da |
| **Average Surface Area:** | > 250$m^2$/g of beads |
| **Average Pore Volume:** | > 1.2mL/g of beads (> 60% of bead volume) |
| **Pore Size:** | 1000Å |
| **Maximum Pressure:** | 100psi (6.9 bar)* |
| **Maximum Linear Velocity:** | 3000cm/hour |

***This value refers to the maximum pressure drop across a column that the resin can withstand. The indicated gauge pressure of a liquid chromatography apparatus may not be measuring the pressure drop across the column.

### B. Calculating the Linear Flow Rate for Medium Pressure Chromatography

An important factor for success when performing medium pressure chromatographic (MPC) applications is limiting the pressure drop across the column, which is critical when attempting to increase scale by using a larger column. The indicated gauge pressure of an MPC apparatus may not actually measure the pressure drop across the column. Therefore, a more reliable criterion for MPC applications is to measure the linear flow rate of buffers through the column, which is a pressure-independent measurement. The linear flow rate is defined as the velocity of the buffer front passing through the resin bed and is usually expressed in cm/hour. UltraLink Resin has a maximum linear flow rate of approximately 3000cm/hour.

The linear flow rate through a cylindrical column can be calculated if the height of the resin bed and the inside diameter (or inside radius) of the column is known, and if column effluent is collected and measured for a given time. The calculations for determining linear velocity are shown below.

**Calculations:**

- r = Radius (cm)
- $\pi r^2$ = Column cross-sectional area
- $1cm^3$ = 1mL of buffer
- $cm^3$/minute = Measured flow rate per minute (i.e., milliliter of effluent collected in 1 minute)

$$\text{Linear velocity/minute} = \frac{cm^3/\text{minute}}{\pi r^2}$$

$$\text{Linear velocity/hour} = (\text{linear velocity/minute})(60\ \text{min/hr})$$

$$\text{therefore, } \frac{(\text{cm}^3/\text{min})(60\ \text{min/hr})}{\pi r^2} = \text{Linear velocity(cm/hr)}$$

### C. Information Available on Our Website

- Tech Tip #7: Remove air bubbles from columns to restore flow rate
- Tech Tip #29: Degas buffers for use in affinity and gel filtration columns
- Tech Tip #43: Protein stability and storage
- Tech Tip #4: Batch and spin cup methods for affinity purification of proteins

## Related Thermo Scientific Products

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| **21440** | **EZ-Link® NHS-PEG Solid Phase Biotinylation Kit: *pre-packed column*** |
| **21450** | **EZ-Link NHS-PEG Solid Phase Biotinylation Kit: *mini-spin columns*** |
| **21430** | **EZ-Link Sulfo-NHS-LC-Biotinylation Kit** |
| **20227** | **Pierce Monomeric Avidin Agarose Kit** |
| **31101** | **NeutrAvidin Protein, Horseradish Peroxidase Conjugated,** 2mg |
| **31002** | **NeutrAvidin Protein, Alkaline Phosphatase Conjugated,** 2mg |
| **15507** | **NeutrAvidin High Binding Capacity Coated Plates**, **96 well, clear**, 5/pkg |
| **66382** | **Slide-A-Lyzer® Dialysis Cassette Kit, 10K MWCO, 3mL**, 10/pkg |
| **43243** | **Polyacrylamide Desalting Columns, 6K MWCO, 10mL, 5/pkg** |

### General References


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