INSTRUCTIONS

Pierce® Streptavidin UltraLink® Resin

53113 53114 53116 53117

0518.8

Number	Description
53113	**Pierce Streptavidin UltraLink Resin,** 2mL settled resin (4mL total volume)
53114	**Pierce Streptavidin UltraLink Resin,** 5mL settled resin (10mL total volume)
	Binding Capacity: ≥ 2mg of biotinylated BSA/mL of resin
	Supplied: 50% aqueous slurry containing 0.02% sodium azide
53116	**Pierce Streptavidin Plus UltraLink Resin,** 2mL settled resin (4mL total volume)
53117	**Pierce Streptavidin Plus UltraLink Resin,** 5mL settled resin (10mL total volume)
	Binding Capacity: ≥ 4mg of biotinylated BSA/mL of resin
	Supplied: 50% aqueous slurry containing 0.02% sodium azide

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

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Introduction

Immobilized streptavidin can be used for affinity chromatography purifications, assay development and immunoprecipitation.[1-3] Immobilized streptavidin also can be used in the physical separation of two DNA strands produced in a polymerase chain reaction by incorporating biotin in one of the amplification polymers.[4]

Streptavidin is similar to avidin, but was originally isolated from culture filtrates of Streptomycetes. Both streptavidin and avidin are rich in tryptophan and highly resistant to denaturation by acids or proteolytic enzymes. Unlike avidin, streptavidin is carbohydrate-free and more resistant than avidin to dissociation into subunits by guanidine•HCl. Streptavidin generally has less nonspecific binding than avidin because of the absence of carbohydrates and the difference in charge.

The Thermo Scientific UltraLink Biosupport is an azlactone-activated polyacrylamide resin that is hydrophilic, charge-free, porous, highly crosslinked and rigid. The physical characteristics (Table 1) of this support are beneficial for large sample volumes requiring fast-flow techniques. Agarose supports are extremely useful for gravity-flow procedures; however, this more rigid support is required if pressures are greater than 25psi UltraLink Biosupport is, therefore, useful for medium-pressure techniques such as FPLC.

Table 1. Characteristics of the Thermo Scientific UltraLink Biosupport.

pH Stability:	1-13
Particle Size (average):	50-80µm
Exclusion Limit (proteins):	> 2,000,000Da
Surface Area (average):	> 250m^2/g of beads
Pore Volume (average):	> 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 biosupport can withstand. The indicated gauge pressure of a liquid chromatography apparatus may not be measuring the pressure drop across the column.

Important Product Information

- Biotinylated molecules can be eluted from the immobilized streptavidin with 8M guanidine•HCl, pH 1.5 (Product No. 24115) or by boiling with SDS-PAGE sample buffer. These harsh elution conditions may cause leaching of the streptavidin subunits into the sample. Additionally, the immobilized streptavidin have a reduced binding capacity from the loss of these subunits.

  Note: These elution conditions may irreversibly damage the protein of interest. If the biotinylated molecule needs to be eluted using non-denaturing conditions, use Thermo Scientific Pierce Monomeric Avidin Agarose (Product No. 20228), which allows for mild elution conditions to recover biotinylated molecules without contamination from streptavidin subunits and without reducing the binding capacity of the purification agarose. Alternatively, biotinylate the protein using Thermo Scientific EZ-Link NHS-Iminobiotin Trifluoroacetamide (Product No. 21117), which binds to streptavidin at pH 9.5 and is easily dissociated at pH 4. Reversible biotinylation may be achieved using a cleavable reagent such as EZ-Link® Sulfo-NHS-SS-Biotin (Product No. 21331).

- The protocols included in these instructions are example applications for this product. Specific applications and systems will require optimization.

Procedure for Batch Format Immunoprecipitation Using Immobilized Streptavidin

A. Additional Materials Required

- Binding Buffer: Phosphate-buffered Saline (e.g., 0.1M 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 ~3mg of biotinylated protein/mL of settled Thermo Scientific Pierce Streptavidin UltraLink Resin (2mL of the 50% slurry is equivalent to 1mL of settled resin) and ~5mg of biotinylated protein/mL of the Pierce Streptavidin Plus UltraLink 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)
- 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 will have to be optimized 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 immobilized streptavidin and reagents to room temperature.

2. To form the immune complex, add biotinylated antibody to the sample or lysate and incubate for at least 30 minutes at room temperature or overnight at 4ºC.
3. Gently swirl the bottle of immobilized streptavidin to obtain an even suspension. Pipette the appropriate amount of resin into a microcentrifuge tube. 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 streptavidin-bound complex with Binding Buffer and centrifuge for 1 minute at medium speed. Discard the supernatant. 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 the 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).

Procedure for Column Format Affinity Purification using Immobilized Streptavidin

A. Additional Materials Required

- Binding Buffer: Phosphate-buffered saline (PBS; 0.1M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372)
- Biotinylated Antibody: Use ~3mg of biotinylated protein/mL of settled Pierce Streptavidin UltraLink Resin (2mL of the 50% slurry is equivalent to 1mL of settled resin) and ~5mg of biotinylated protein/ml of the Pierce Streptavidin Plus UltraLink Resin. Prepare biotinylated antibody at 0.2-10mg/mL in Binding Buffer.
- Sample containing antigen of interest
- Elution Buffer: IgG Elution Buffer (Product No. 21004), Gentle Ag/Ab Elution Buffer (Product No. 21027) or 0.1M glycine•HCl, pH 2.8
- Columns: Disposable Polystyrene Columns: Product No. 29920 for resin volumes of 2mL or less or Product No. 29924 for resin volumes of 2-10mL

B. Procedure

1. Equilibrate the immobilized streptavidin and reagents to room temperature.
2. Pack resin into the column according to the product instructions. When using packed columns, remove the top cap first, empty the storage solution and then remove bottom cap, which prevents air bubbles from being drawn into the resin.
3. Equilibrate column by adding five column volumes of Binding Buffer and allowing the solution to drain through.
4. Add biotinylated antibody to the column and allow solution to enter the resin bed. Replace the bottom and top caps sequentially and incubate at room temperature for 30 minutes.

   Note: If the solution volume is such that the entire sample cannot be added at once, incubate for 10-15 minutes and allow some of the solution to pass through the column. Then add more antibody solution and incubate.
5. Wash the column with 10 column volumes of Binding Buffer.
6. Add antigen sample to the column and allow the solution to enter the resin bed. Replace the bottom and top caps sequentially and incubate at room temperature for 30 minutes or overnight at 4ºC.
7. Wash the column with 10 column volumes of Binding Buffer.
8. 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 to 1mL of the 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 antigen elution. The Gentle Elution Buffer is not compatible with phosphate-based buffers.
9. Desalt or dialyze the eluted fractions into a buffer suitable for the downstream application.

10. To reuse the immobilized biotinylated antibody to purify more antigen, wash column with 10 column volumes of Binding Buffer, add a final concentration of 0.02% sodium azide and store at 4°C.

Procedure for Gravity-Flow Purification of Biotinylated Molecules

A. Additional Materials Required

- Binding Buffer: Phosphate-buffered saline (PBS; 0.1M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372)
- Biotinylated sample in solution: Use ~3mg of biotinylated protein/mL of settled Pierce Streptavidin UltraLink Resin (2mL of the 50% slurry is equivalent to 1mL of settled resin) and ~5mg of biotinylated protein/mL of the Pierce Streptavidin Resin UltraLink Resin. Prepare biotinylated antibody at 0.2-10mg/mL in Binding Buffer.
- Elution Buffer: 8M guanidine•HCl, pH 1.5 (Product No. 24115)
- Columns: Disposable Polystyrene Columns (Product No. 29920 for resin volumes of 2mL or less or Product No. 29924 for resin volumes of 2-10mL)

B. Procedure

1. Equilibrate the immobilized streptavidin and reagents to room temperature.
2. Pack the resin into the column according to the instructions provided with the columns. When using packed columns, remove the top cap first, empty the storage solution and then remove bottom cap, which prevents air bubbles from being drawn into the resin.
3. Equilibrate the column by adding three column volumes of Binding Buffer and allowing the solution to drain through.
4. Add biotinylated sample to the column and allow sample to enter the resin bed. Sequentially replace the bottom and top caps and incubate at room temperature for 30 minutes.

   Note: If the solution volume is such that the entire sample cannot be added at once, incubate for 10-15 minutes and allow some of the solution to pass through the column. Then add more antibody solution and incubate. Do not exceed the resin's binding capacity.
5. Wash the column with 10 column volumes of Binding Buffer.
6. Elute the bound biotinylated sample with 5-10 column volumes of Elution Buffer. Collect the eluate in 0.5-1mL fractions. Monitor protein content by measuring the absorbance of each fraction at 280nm.
7. Immediately desalt or dialyze the eluted fractions of interest.

Procedure for Medium-Pressure Chromatography Purification of Biotinylated Molecules

A. Additional Materials Required

- Biotinylated sample in solution: Use ~3mg of biotinylated protein/mL of settled Pierce Streptavidin UltraLink Resin (2mL of the 50% slurry is equivalent to 1mL of settled resin) and ~5mg of biotinylated protein/mL of the Pierce Streptavidin Plus UltraLink Resin. Prepare biotinylated antibody at 0.2-10mg/mL in Binding Buffer.
- Binding Buffer: Phosphate-buffered saline (PBS; 0.1M phosphate, 0.15M NaCl; pH 7.2; Product No. 28372)
- Elution Buffer: 8M guanidine•HCl, pH 1.5 (Product No. 24115)
- Medium-Pressure Chromatography column (see Additional Information Section for calculations)

B. Procedure

1. Gravity pack or pack under flow the streptavidin resin into a medium-sized pressure chromatography column. Store the column in 0.02% sodium azide if column will not be used immediately.
2. Equilibrate the column with at least three column volumes of the binding buffer.
3. Add or inject the biotinylated sample. Wash the column with eight column volumes of Binding Buffer or until baseline absorbance (280nm) is reached.

4. Elute the bound biotinylated protein with 15 column volumes of Elution Buffer or until baseline is reached. Collect 0.5-1mL fractions. Monitor the elution of the biotinylated protein by measuring the absorbance at 280nm.
5. Dialyze or desalt the eluted biotinylated protein.

Additional Information

A. 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 Biosupport 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)
- πr^2 = Column cross-sectional area
- 1 cm^3 = 1mL of buffer
- cm^3/minute = Measured flow rate per minute (i.e., milliliter of effluent collected in 1 minute)

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

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

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

B. Information Available on Our Website

Please visit our web site for additional information relating to this product including the following items:

- Tech Tip #27: Optimize elution conditions for immunoaffinity purification
- 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 #13: Pack beaded affinity resin into columns

Related Thermo Scientific Products

21435	**EZ-Link® Sulfo-NHS-LC-Biotinylation Kit**
21440	**EZ-Link NHS-PEG Solid Phase Biotinylation Kit: *pre-packed column***
21450	**EZ-Link NHS-PEG Solid Phase Biotinylation Kit: *mini-spin columns***
29200	**NeutrAvidin™ Agarose Resin,** 5mL
20227	**Pierce Monomeric Avidin Agarose Kit**
21224	**Streptavidin, Fluorescein (FITC) Conjugated,** 1mg

21724	**Streptavidin, Rhodamine (TRITC) Conjugated,** 1mg
66425	**Slide-A-Lyzer® Dialysis Cassettes, 10K MWCO, 3mL,** 10/pkg
43243	**Polyacrylamide Desalting Columns, 6K MWCO, 10mL**, 5/pkg

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