INSTRUCTIONS

UltraLink® Hydrazide Resin

Thermo SCIENTIFIC

53149 0514.5

Number	Description
53149	**UltraLink Hydrazide Resin**, 10mL resin, 20mL slurry
	Support: UltraLink Biosupport (copolymer of *bis*-acrylamide and azlactone)
	Supplied as a 50% slurry in 0.02% sodium azide
	Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

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Introduction

The Thermo Scientific UltraLink Hydrazide Resin is an affinity support for immobilizing glycoproteins through oxidized sugar groups. It is ideal for immobilizing polyclonal antibodies, which contain abundant carbohydrates located on the Fc portion of the molecule. Because such antibodies are coupled to the UltraLink Hydrazide Resin through the Fc portion only, they are properly oriented with their antigen-binding sites unobstructed, offering greater purification capability. Some monoclonal antibodies may be immobilized using UltraLink Hydrazide Resin, provided they contain an adequate amount of carbohydrate.[1]

The immobilization chemistry uses sodium *meta*-periodate to oxidize *cis*-glycol groups in sugars of polysaccharide moieties. The resulting aldehydes then react spontaneously with hydrazide groups on the UltraLink Hydrazide Resin to form stable hydrazone bonds. The coupling conditions are flexible with regard to time and temperature. The long spacer arm (Figure 1) reduces steric hindrance and the support has minimal nonspecific binding characteristics, making this an excellent resin for affinity chromatography. When coupled to stable glycoproteins, columns may be regenerated and reused at least 10 times.

UltraLink Biosupport is a rigid, highly crosslinked, copolymeric and porous resin with high coupling capacity.[2-8] The charge-free support has minimal nonspecific interactions with most sample types. The porosity, rigidity and durability of UltraLink Biosupport make it suitable for medium-pressure, fast-flow techniques involving large sample volumes. When packed into a 3mm diameter × 14cm height column, UltraLink Biosupport affinity columns have been run to approximately 400psi (system pressure) with no visual compression of the resin or adverse effects on chromatography. Typically, UltraLink Biosupport columns can be used with linear flow rates of 85-3000cm/hour with excellent separation characteristics. (See the Additional Information Section of these instructions.) By contrast, crosslinked beaded agarose supports are only suitable for gravity-flow procedures involving < 25psi.

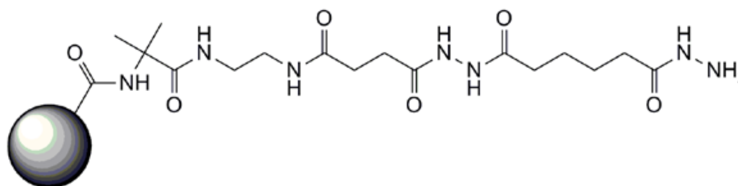

Figure 1. Spacer arm structure of Thermo Scientific UltraLink Hydrazide Resin.

Procedure for Immobilizing a Glycoprotein

Note: This procedure assumes the use of 2mL of UltraLink Hydrazide Resin and a 5mL gravity-flow desalting column to prepare and couple 0.5-10mg of glycoprotein. To immobilize glycoprotein at a smaller or larger scale, adjust the procedure accordingly.

A. Additional Materials Required

- Coupling Buffer: 0.1M sodium phosphate buffer, pH 7.0. Different buffers may be used, but avoid those containing primary amines (e.g., Tris) or sugars, which will compete with the intended coupling reaction. For optimal coupling efficiency, use Thermo Scientific GlycoLink Coupling Catalyst (Product No. 88944) and follow the included catalyst instructions.
- Oxidizing Agent: Sodium *meta*-Periodate (Product No. 20504)
- Amber glass vial or other tube that will protect the oxidation reaction from light
- Desalting column (e.g., Thermo Scientific Zeba Spin Desalting Columns, Product No. 89891)
- Empty gravity-flow or spin column that may be capped both top and bottom: (e.g., Product No. 29925) see Section C
- Wash Solution: 1M NaCl

B. Prepare Glycoprotein Sample for Coupling

Note: Coupling efficiency is significantly improved when using GlycoLink™ Coupling Catalyst (Product No. 88944).

Note: Steps 2 and 3 are light-sensitive and must be performed in an amber vial.

1. Dissolve 0.5-10mg antibody or other glycoprotein in Coupling Buffer. If the protein is already dissolved in an incompatible buffer (see Additional Materials Required), perform a buffer exchange using a desalting column or dialysis.

   Note: Do not exceed 1mL for the sample volume if a 5mL gravity-flow desalting column will be used in steps 6-8.
2. Weigh 2.14-5.35mg of sodium *meta*-periodate (Oxidizing Agent) into a small amber vial. (This weight of reagent results in 10-25mM periodate when dissolved in the 1mL glycoprotein sample from step 1.)
3. Add the protein solution to the amber vial containing the Oxidizing Agent, gently swirling the vial until the powder is dissolved.
4. Incubate the sample for 30 minutes at room temperature. Perform step 5 during this incubation.
5. Equilibrate a 5mL Zeba™ Spin Desalting Column (Product No. 89891) with Coupling Buffer by following the instructions included with the desalting column.
6. Apply the oxidized sample (~1mL) to the equilibrated Zeba Spin Desalting Column. Centrifuge the column for 2 minutes at 1000 × *g* to collect the desalted sample.
7. Record the A_{280} measurement of the protein sample for later calculation of the coupling efficiency (see Step D4).

C. Couple Oxidized Glycoprotein to UltraLink Hydrazide Resin

Note: Packing the UltraLink Resin into a gravity-flow or spin column makes it easy to perform the coupling reaction as a sample-resin batch mixture and to quickly wash and recover nonbound sample volumes for analysis of coupling efficiency. Pierce Disposable Plastic Columns (Product No. 29925) with only the bottom disc inserted are useful for this purpose. Other kinds of reaction vessels may be used as long as they allow for effective washing and sample incubation (coupling reaction) steps. This procedure assumes use of a gravity-flow column as the reaction vessel.

1. With the bottom cap in place on a gravity-flow column, add the desired quantity of the UltraLink Hydrazide Resin slurry (e.g., 4mL of slurry containing 2mL of resin) and allow the resin to settle for 15 minutes.
2. Drain the liquid from the packed column and wash/equilibrate the UltraLink Hydrazide Resin with 5 resin-bed volumes of Coupling Buffer by adding buffer to the top of the resin bed and allowing it to drain through the column.

   Note: Throughout the entire procedure, do not allow the resin bed to run dry; instead, add additional solution or replace the bottom cap on the column whenever the solution drains down to the top of the resin bed.

3. Replace the bottom cap and add the oxidized glycoprotein sample to the resin (~1mL of oxidized protein per mL of resin).
4. Replace the top cap and mix the column contents by inverting the column until the resin is in suspension.
5. Mix the column with gentle side-to-side or end-over-end motion for 6 hours at room temperature (vigorous mixing may cause protein precipitation or aggregation). Incubation may be carried out overnight if it is more convenient.

D. Calculate Coupling Efficiency

1. Stand the column upright and allow the resin to settle for 15 minutes.
2. Uncap the column and drain the liquid into a clean collection tube. Remember not to let the resin bed run dry.
3. Wash the column with 1 resin-bed volume of Coupling Buffer and drain into the same collection tube as above.
4. Measure the A_{280} of the collected solution. Accounting for the dilution of the original sample, compare this measurement to that of the starting material (step B9) to estimate the coupling efficiency.

E. Pack, Wash and Store Prepared UltraLink Affinity Column

1. (Optional) Resuspend and pack the prepared UltraLink Affinity Resin into the desired affinity column system.
2. Wash the column with 5 resin-bed volumes of 1M NaCl (Wash Solution).
3. Wash the column with 5 resin-bed volumes of a degassed storage buffer such as phosphate buffered saline (PBS) containing 0.05% sodium azide.

The protein is now covalently coupled to the resin through carbohydrate groups and can be used for affinity chromatography. Store the capped column upright at 4°C, or proceed with the General Protocol for Affinity Purification.

General Protocol for Affinity Purification of Protein

A. Sample Purification

1. Equilibrate column by washing it with 5 resin-bed volumes of binding buffer, such as phosphate buffered saline (PBS). Use the same buffer in which the sample is diluted and the target molecule is assumed to bind most efficiently.

   Note: All buffers to be run through a column should be degassed to avoid introducing air bubbles.
2. Apply sample to the column and allow it to completely enter the resin bed.

   Note: For samples that are greater than the resin-bed volume, repeat steps A.1 and A.2 with aliquots equal to the resin bed volume until the entire sample has been used. Alternatively, adjust column flow to allow sufficient binding time for the sample that is being passed through the column.
3. Allow the column to incubate at room temperature for 1 hour.
4. Wash the column with 5 resin-bed volumes of binding buffer.
5. Elute the bound protein by applying 5 resin-bed volumes of IgG Elution Buffer (Product No. 21004 or 21009) or glycine (100mM, pH 2.5-3.0), collecting separate fractions equal to half the resin-bed volume.

   Note: Fractions can be neutralized by adding one-tenth volume of 1M Tris, pH 7.5.
6. Identify fractions containing protein by measuring for those having peak absorbance at 280nm.
7. Pool fractions of interest and exchange into an appropriate storage buffer by desalting or dialysis.

B. Column Regeneration and Storage

Note: Regenerate the column as soon as possible after chromatography to prevent damage to the immobilized molecule by the low pH elution buffer.

1. Wash column with 5 resin-bed volumes of binding buffer to remove all elution buffer.
2. Equilibrate column with 5 resin-bed volumes of an appropriate degassed buffer containing 0.05% sodium azide.
3. Store capped column in buffer at 4°C.

Troubleshooting

Problem	Cause	Solution
Low coupling efficiency	Protein is not heavily glycosylated or the sugars are not oxidizable	Choose another immobilization method
	Primary amines were not removed from the sample before coupling and competed for binding to the protein	Ensure primary amines have been thoroughly removed by dialysis or desalting
		Reduce small peptides using Thermo Scientific Immobilized TCEP Disulfide Reducing Gel (Product No. 77712) to avoid contamination of sample with reductant
Column flows very slowly	Air bubbles in column were restricting flow (air bubbles may not be visible)	Remove air bubbles by stirring or centrifugation (See Tech Tip #7: Remove air bubbles from columns to restore flow rate) on the website
Affinity column loses binding capacity over time	Immobilized sample had been damaged by time, temperature or elution conditions	Prepare a new affinity column and alter the procedure responsible for damage to the column
	Column had become fouled with non-specifically bound material	Wash column with high salt (e.g., ~1M NaCl) to remove nonspecifically bound material
		Prepare a new affinity column. Remove foulants from sample before affinity purification

Related Thermo Scientific Products

20504 **Sodium *meta*-Periodate**, 25g

88944 **GlycoLink Coupling Catalyst,** 100mL

28020 **EZ-Link® Hydrazide Biocytin,** 25mg

21339 **EZ-Link Hydrazide Biotin,** 100mg

21340 **EZ-Link Hydrazide-LC-Biotin,** 50mg

22106 **EMCH,** *N*-(ε-maleimidocaproic acid)hydrazide, 50mg

22111 **KMCH,** *N*-(κ-maleimidoundecanoic acid)hydrazide, 50mg

22305 **MPBH,** 4-(4-*N*-maleimidophenyl)butyric acid hydrazide, 50mg

53155 **UltraLink Iodoacetyl Resin**, 10mL

21004 **IgG Elution Buffer**, 1L

28372 **BupH™ Phosphate Buffered Saline Packs**, 40 packs

Additional Information

A. Please visit the web site for additional information on 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

B. Medium-pressure Chromatographic Applications

Because UltraLink Biosupport can withstand pressures up to 100psi (Table 1), it can be used in medium pressure chromatographic (MPC) applications. Affinity procedures with UltraLink Biosupport generally perform well using linear velocities up to 3000cm/hour for a 1cm diameter × 10cm high resin bed. Linear velocity is the rate at which the buffer front passes through the resin bed. The linear velocity through a cylindrical column can be calculated if the inside diameter of the column is known and the volume of column effluent per time is measured to determine the flow rate. The calculations for determining linear velocity are as follows:

Radius measured in cm = r
Column cross-sectional area in square centimeters = πr^2
1mL = 1 cubic centimeter = $1cm^3$
Measured flow rate in cubic centimeters per minute = (mL of column effluent collected per minute)($1cm^3$/mL)
Linear velocity expressed as centimeters per hour = $\frac{\text{(measured flow rate)(60 minutes/hour)}}{\text{column cross-sectional area in cm}^2}$

Table 1. Characteristics of the Thermo Scientific UltraLink Biosupport.

Characteristic	Specification
pH stability of matrix	1-13
Particle size (average)	50-80 microns
Exclusion limit (proteins)	> 2,000,000 daltons
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 (6.0 bar)*
Maximum linear velocity	3000cm/hour

* The indicated maximum pressure of 100psi refers to the maximum pressure-drop across a column that the support can withstand. It does not necessarily refer to the indicated system pressure shown on a liquid chromatography apparatus, because the system pressure may not be actually measuring the pressure drop across the column.

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