INSTRUCTIONS

UltraLink® Iodoacetyl Gel

53155

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Number	Description
53155	**UltraLink Iodoacetyl Gel,** 10mL settled gel, supplied as 50% slurry (20mL total volume) in 1M sodium chloride and 0.02% sodium azide Binding Capacity: ≥ 6mg of reduced human IgG/mL of settled gel

Storage: Upon receipt store product at 4°C, protected from light. Product is shipped at ambient temperature.

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Introduction

The Thermo Scientific UltraLink Iodoacetyl Gel enables simple and efficient covalent immobilization of sulfhydryl-containing peptides, proteins and other ligands to a gel support for use in affinity purification procedures. The gel reacts specifically with free sulfhydryls to form a stable thioether linkage. The support contains a 15-atom spacer that reduces steric hindrance, making binding interactions with the coupled molecule efficient. Average coupling efficiencies are greater than 80%, although efficiency is related to sulfhydryl content and is variable. The gel is ideal for conjugating sulfhydryl-containing peptide for subsequent antibody purification.

The UltraLink Biosupport is a rigid, highly crosslinked, co-polymeric and porous resin with high coupling capacity. 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 tall column, UltraLink Biosupport affinity columns have been used with approximately 400 psi (system pressure) with no visual compression of the gel 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 Additional Information section). By contrast, crosslinked beaded agarose supports are only suitable for gravity-flow procedures involving < 25 psi.

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Important Product Information

The peptide or protein to be immobilized must have free (reduced) sulfhydryls. Synthesized peptides, if used immediately after reconstitution, usually can be assumed to have reduced sulfhydryls appropriate for coupling. Ellman's Reagent (Product No. 22582) can be used to determine if the peptide/protein contains free sulfhydryls. To make free sulfhydryls, cleave disulfide bonds with a reducing agent. If a sulfhydryl-containing reducing agent was used, desalting or dialysis must be performed to remove the reducing agent before immobilization.

- **Peptide samples:** Tris(2-carboxyethyl)phosphine (TCEP, Product No. 77720), efficiently reduces peptides but does not interfere with iodoacetyl coupling, requiring no removal of excess reagent before immobilization. TCEP is stable in aqueous solution and selectively reduces disulfide bonds.

  Dissolve or dilute 0.1-1mg of peptide in 2mL of Coupling Buffer and add TCEP to a final concentration of 25mM TCEP. Note that TCEP interferes with the Thermo Scientific Pierce BCA Protein Assay.

- **Antibody samples:** Reduce disulfide bonds using 2-mercaptoethylamine•HCl (2-MEA, Product No. 20408). 2-MEA can selectively cleave hinge-region disulfide bonds between IgG heavy chains while preserving the disulfide bonds between the heavy and light chains. The result is two half antibodies with sulfhydryls available for immobilization.

  Dissolve or dilute 1-10mg of protein with 1mL of buffer (0.1M sodium phosphate, 5mM EDTA-Na; pH 6.0). Add the protein solution to 6mg of 2-MEA (50mM). Incubate mixture at 37°C for 1.5 hours. Remove 2-MEA by performing two passes through a Thermo Scientific Zeba Spin Desalting Column (see Related Thermo Scientific Products) using the Coupling Buffer.

Procedure for Coupling to UltraLink Iodoacetyl Gel

A. Additional Materials Required

- Coupling Buffer: 50mM Tris, 5mM EDTA-Na; pH 8.5. Prepare a volume of buffer equal to 20 times the volume of UltraLink Iodoacetyl Gel to be used.
- L-Cysteine•HCl: Product No. 44889
- Wash Solution: 1M sodium chloride (NaCl)
- Storage Buffer: Phosphate buffered saline (PBS) (e.g., BupH™ Phosphate Buffered Saline Packs containing 0.1M phosphate, 0.15M NaCl, pH 7.2, Product No. 28372), or other suitable buffer containing 0.02% sodium azide
- Empty gravity-flow or spin column that many be capped both top and bottom: (e.g., Product No. 29925) see Section C

  Note: For spin-column formats, use the Pierce Mini-Spin Columns and Accessories (Product No. 69705).
- Elution Buffer for affinity purification: IgG Elution Buffer (Product No. 21004 or 21009) or 100mM glycine at pH 2.5-3.0

B. Prepare the Peptide or Protein Sample

1. Dissolve the sample with Coupling Buffer. If the sample is not soluble in Coupling Buffer, dissolve it in a suitable buffer at pH 8-8.5 (see the Troubleshooting Section). Dilute samples already in solution 1:1 in Coupling Buffer.

   Note: If the sample is in a buffer that contains compounds with free sulfhydryls (e.g., 2-mercaptoethanol or DTT), these compounds will quench the coupling reaction and must be thoroughly removed by dialysis or desalting.

C. Couple to UltraLink Iodoacetyl Gel

Note: Packing the UltraLink Gel into a gravity-flow or spin column makes it easy to perform the coupling reaction as a sample-gel 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 Iodoacetyl Gel slurry (e.g., 4mL slurry containing 2mL of gel) and allow the gel to settle for 15 minutes.
2. Drain the liquid from the packed column and wash/equilibrate the UltraLink Iodoacetyl Gel with five gel-bed volumes of Coupling Buffer by adding buffer to the top of the gel bed and allowing it to drain through the column.

Note: Throughout the entire procedure, do not allow the gel 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 gel bed.

3. Replace bottom cap and add the prepared sulfhydryl-containing sample. Approximately 1mL of sample solution can be applied per milliliter of UltraLink Iodoacetyl Gel. To determine coupling efficiency, reserve a portion of the sample for comparison to the non-bound fraction that will be collected in Step 8.
4. Replace the top cap and mix column at RT for 15 minutes.
5. Stand the column upright and incubate at RT for 30 minutes without mixing.
6. Sequentially remove top and bottom column caps and allow the solution to drain.
7. Wash column with three gel-bed volumes of Coupling Buffer.
8. Determine the coupling efficiency by comparing the protein/peptide concentrations (e.g., by absorbance at 280nm) of the non-bound fraction to the starting amount.

D. Block Nonspecific Binding Sites on Gel

1. Replace the bottom cap on column.
2. Prepare a solution of 50mM L-Cysteine•HCl in Coupling Buffer and add 1mL of this solution to the column for each milliliter of gel.
3. Replace the top cap and mix for 15 minutes at RT, then incubate the reaction without mixing for an additional 30 minutes at RT.

E. Pack, Wash and Store Prepared UltraLink Affinity Column

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

   The sample molecule is now covalently coupled to the gel through sulfhydryl 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

A. Sample Purification

1. Equilibrate column by washing it with five gel-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 for column use should be degassed to avoid introducing air bubbles.

2. Apply sample to the column and allow it to completely enter the gel bed.

   Note: For samples that are greater than the gel-bed volume, repeat steps A.2 and A.3 with amounts equal to the gel 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 five gel-bed volumes of binding buffer.
5. Elute the bound protein by applying five gel-bed volumes of IgG Elution Buffer (Product No. 21004 or 21009) or glycine buffer (100mM, pH 2.5-3.0), collecting separate fractions equal to half the gel-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 five gel-bed volumes of binding buffer to remove all elution buffer.
2. Equilibrate column with five gel-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
Protein/peptide precipitates in Coupling Buffer	Protein/peptide was not soluble in Coupling Buffer	Dissolve sample in ≤ 30% DMSO or DMF or 6M guanidine•HCl
Low coupling efficiency	Sulfhydryls were not reduced	Reduce protein/peptide and proceed immediately with desalting and coupling procedure to avoid reformation of disulfide bonds
	Sulfhydryl-containing reductant was not removed from sample	Remove reductant from the reduced sample using a desalting column before immobilization
		Reduce peptides using TCEP or Immobilized TCEP Disulfide Reducing Gel (Product No. 77712)
Gravity-flow column flows exceedingly slow	Air bubbles in column restricted flow (air bubbles may not be visible)	Remove air bubbles by stirring or centrifugation. (See Tech Tip Protocol "Remove Air Bubbles From Columns" on our website)
Affinity column loses binding capacity over time	Immobilized sample was damaged by time, temperature or elution conditions	Prepare a new affinity column and alter the condition responsible for damaging the column
	Column was fouled with nonspecifically bound material	Prepare a new affinity column and remove foulants from sample before affinity purification

Additional Information

A. Specific Physical Characteristics of the UltraLink Biosupport

The UltraLink Biosupport 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 Biosupport.

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

*This value refers to the maximum pressure drop across a column that the gel 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 gel 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 gel 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
- $1cm^3$ = 1mL of buffer
- cm^3/minute = Measured flow rate per minute (i.e., milliliter of effluent collected in 1 minute)

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

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

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

C. Information Available from the Internet

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

- Tech Tip Protocol: Remove Air Bubbles from Columns
- Tech Tip Protocol: Degas Solutions for use in Affinity Columns
- Tech Tip: Protein Stability and Storage
- Tech Tip Protocol: Batch and Spin Cup Methods for Affinity Purification of Proteins

Related Thermo Scientific Products

44999	**SulfoLink Immobilization Kit for Peptides**
44995	**SulfoLink Immobilization Kit for Proteins**
77720	**Bond-Breaker TCEP Solution, Neutral pH,** 5mL
20408	**2-Mercaptoethylamine•HCl (2-MEA)**, 6 × 6mg in amber screw-cap vials
20401	**SulfoLink Coupling Resin,** 10mL
89891	**Zeba Spin Desalting Columns, 5mL,** 5 columns, for 500-2000µL samples
89892	**Zeba Spin Desalting Columns, 5mL,** 25 columns, for 500-2000µL samples
28372	**BupH Phosphate Buffered Saline Pack,** 40 packs
22582	**Ellman's Reagent,** 5g
69700	**Pierce Spin Cup Columns**
29925	**Disposable Column Trial Pack,** 6 columns (two of each size) plus accessories

General References for UltraLink Biosupport

Coleman, P.L., *et al.* (1988). Affinity chromatography on a novel support: azlactone-acrylamide copolymer beads. *FASEB J* **2**:A1770 (#8563).

Coleman, P.L., *et al.* (1990). Azlactone copolymer beads: applications in bioseparations. *J Cell Biochem* **44:**19 (S14D).

Hermanson, G.T., *et al.* (1992). Immobilized Affinity Ligand Techniques. Academic Press, Inc., pp. 28-31, 90-95. (Available as Product No. 22230.)

Ju, T., *et al.* (2002). Purification, characterization, and subunit structure of rat core 1 ß1,3-galactosyltransferase. *J Biol Chem* **277(1):**169-77.

Product References for UltraLink Iodoacetyl Gel

Bicknell, A.B., *et al.* (2001). Characterization of a serine protease that cleaves pro-γ-melanotropin at the adrenal to stimulate growth. *Cell.***105:**903-12.

Liu, L.A. and Engvall, E. (1999). Sarcoglycan isofoms skeletal muscle. *J Biol Chem* **274(53):**38171-6.

Magdesian, M.H., *et al.* (2001). Infection by *Trypanosoma cruzi*. *J Biol Chem* **276(22):**19382-9.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.