INSTRUCTIONS

Protein G UltraLink® Resin

0495.8

Number	Description
53125	**Protein G UltraLink Resin**, 2mL settled resin
53126	**Protein G UltraLink,** 10mL settled resin Support: Supplied as a 50% slurry (e.g., 2mL of settled resin is equivalent to 4mL of 50% slurry) containing 0.02% sodium azide Binding capacity: > 20mg human IgG/mL resin
53127	**Protein G UltraLink Columns**, 2 × 2 mL resin (50% slurry in 0.02% sodium azide) pre-packed in columns with twist-off bottom tabs and an accessory pack containing two white tips Column binding capacity: > 40 mg human IgG
53128	**Protein G Plus UltraLink Resin**, 2mL settled resin Support: Supplied as a 50% slurry (e.g., 2mL of settled resin is equivalent to 4mL of 50% slurry) containing 0.02% sodium azide Binding capacity: > 25mg human IgG/mL resin

Storage: Upon receipt store product at 4°C. Products are shipped at ambient temperature.

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Introduction

The Thermo Scientific Protein G UltraLink and Protein G Plus Ultralink Resins are useful for purification of antibodies and immunoprecipitation (IP). Samples containing IgG are incubated with the resin-immobilized Protein G in a buffer that facilitates binding. After non-IgG and non-antigen components of the sample are washed from the resin, the bound IgG and antigen may be recovered by elution.

Protein G, a bacterial cell wall protein isolated from group G *Streptococci*,[1-4] binds to mammalian IgGs mainly through Fc regions.[4] Native Protein G has two IgG binding domains and also sites for albumin and cell-surface binding.[5-10] Albumin and cell-surface binding domains have been eliminated from recombinant Protein G to reduce nonspecific binding. Consequently, recombinant Protein G can be used to separate IgG from crude human or mouse IgG serum samples.

Although the tertiary structures of Protein G and Protein A are similar, their amino acid compositions differ significantly, resulting in different binding characteristics.[7-10] Protein G has greater affinity than Protein A for most mammalian IgGs, especially for certain subclasses including human IgG_3, mouse IgG_1 and rat IgG_{2a}.[1,2,3,4,7] Unlike Protein A, Protein G does not bind to human IgM, IgD and IgA.[3,7,10]

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The UltraLink Biosupport is an azlactone-activated support that is hydrophilic, charge-free, high capacity, highly cross-linked, rigid, copolymeric and porous. This support is especially useful for medium pressure techniques when using large sample volumes requiring fast-flow techniques (FPLC) and large-scale applications. Agarose supports are useful for gravity-flow procedures; however, more rigid UltraLink Biosupport is required if flow rates require pressures greater than 25psi. More specific information regarding this support is detailed in the Additional Information Section.

Important Product Information

- Optimal binding to Protein G is dependent upon buffer composition. Thermo Scientific Pierce Binding and Elution Buffers have been optimized to provide the highest efficiency of IgG binding and elution for most species. Using other buffer formulations may significantly alter the binding capacity and the wash volumes required for efficient purification and, therefore, optimization may be necessary. See Product Description Section on page 1 for binding capacities.
- For optimal recovery, use a sample size such that the expected IgG load is less than 80% of the maximum binding capacity. The total IgG content of serum is approximately 10-15mg/mL. The concentration of antibody in tissue culture supernatant varies considerably among hybridoma clones. Be aware that antibodies from fetal bovine serum (FBS) culture media supplement will be purified along with the antibody of interest.

Column Procedure for Antibody Purification Using Protein G UltraLink Resin

Note: The following protocol is for using a gravity-flow column packed with 1mL of Protein G UltraLink Resin (i.e., 2mL of the 50% slurry). When using columns containing other resin volumes, reagent amounts must be adjusted accordingly. For example, double all volumes when using the 2mL pre-packed columns in Product No. 53127. See the Additional Information Section for batch and spin cup methods.

A. Additional Materials Required

- Pierce® Centrifuge Columns (e.g., Product No. 89897). These columns are for gravity-flow or centrifuge methods.
- Binding Buffer: Protein G IgG Binding Buffer (Product No. 21011)
- Elution Buffer: IgG Elution Buffer (Product No. 21004 or 21009) or 0.1M glycine, pH 2-3
- Neutralization Buffer: 1mL of high-ionic strength alkaline buffer such as 1M phosphate or 1M Tris (pH 8-9)
- (Optional): Thermo Scientific Slide-A-Lyzer Dialysis Cassette or Zeba Spin Desalting Columns (Product No. 89893) for buffer exchange

B. Antibody Purification Procedure

1. Equilibrate Protein G UltraLink Resin and all buffers to room temperature.
2. Carefully pack the column with 2mL of resin slurry, following the instructions provided with the columns.
3. Equilibrate the column by adding 5mL of the Binding Buffer and allowing the solution to drain through the column.

   Note: To avoid air bubbles being drawn into the resin bed, remove top cap before the bottom cap when opening column. The pre-packed column (Product No. 53127) has a twist-off bottom tab; recap column bottom with a supplied white tip.
4. Dilute sample at least 1:1 with Binding Buffer before application to the Protein G Column to maintain the proper ionic strength and pH for optimal binding.

   Note: Plasma may become hazy upon dilution with the Binding Buffer because of lipoprotein precipitation. Centrifuge the diluted sample at 10,000 × *g* for 20 minutes and apply the supernatant to the equilibrated Protein G UltraLink.
5. Apply the diluted sample to the column and allow it to flow completely into the resin bed. Do not allow the resin bed to run dry. Any volume may be applied provided the total amount of antibody is less than 80% of column capacity.

   Note: If the sample contains more IgG than can bind to the Protein G column (or is an antibody type that does not bind to Protein G), the flow-through will contain excess antibody. By saving the flow-through, non-bound antibody can be recovered and examined by antibody-specific assays.
6. Wash the Protein G column with 15mL of the Binding Buffer.

Note: If desired, verify that all non-bound proteins are removed from the column by collecting separate 2mL fractions as the solution drains and measuring their absorbance at 280nm. The last fractions should have absorbances similar to Binding Buffer alone.

7. Elute antibodies with 5mL of Elution Buffer and collect 0.5-1mL fractions. Immediately adjust eluted fractions to physiologic pH by adding 100µL of the Neutralization Buffer per 1mL of eluate. Monitor the elution by measuring the absorbance at 280nm or by protein assay such as the Thermo Scientific Pierce BCA Protein Assay Kit (Product No. 23225).
8. Pool the eluted IgG fractions that contain the highest absorbance. The purified antibodies may be used directly for SDS-PAGE, or the buffer may be exchanged by dialysis or desalting column to one that is compatible with the specific downstream application (see Related Thermo Scientific Products).
9. Regenerate column by washing with 12mL of Elution Buffer. Columns may be regenerated at least 10 times without significant loss of binding capacity.
10. For storage, wash column with 5mL of 0.02% sodium azide (in water). When approximately 3mL of solution remains, replace the bottom cap followed by the top cap on the column. Store columns upright at 4°C.

Example Immunoprecipitation (IP) Procedure Using Protein G UltraLink Resin

A. Additional Materials Required

- 1.5-2mL microcentrifuge tube
- IP Buffer: 25mM Tris, 150mM NaCl; pH 7.2 (Thermo Scientific BupH Tris Buffered Saline Pack, Product No. 28379)
- Antigen Sample: Antigen-containing lysate or sample prepared in IP Buffer or other buffer that is compatible with both the desired antibody binding interaction and the binding of antibody to Protein G
- Elution Buffer: IgG Elution Buffer (Product No. 21004) or 0.1-0.2M glycine•HCl buffer, pH 2.5-3.0
- Electrophoresis Loading Buffer: Lane Marker Reducing Sample Buffer (5X), (Product No. 39000)
- Neutralization Buffer (optional): 1mL of strong alkaline buffer, such as 1M phosphate or 1M Tris, (pH 7.5-9)

B. Immunoprecipitation Procedure

Note: This procedure uses 50µL of settled Protein G UltraLink Resin (100µL resin slurry). This amount of resin is sufficient to bind 25-250µg of antibody. Depending on the amount of antibody needed to immunoprecipitate the desired amount of antigen, scale the amount of resin and suggested wash and elution volumes accordingly. To allow for proper mixing, make sure the total reaction volume does not completely fill the microcentrifuge tube.

1. In a microcentrifuge tube, combine 50-1000µL of the Antigen Sample and the optimized amount of antibody. Incubate the reaction overnight at 4°C.
2. Add 100µL of Protein G Ultralink Resin slurry to the antigen-antibody complex.
3. Incubate reaction with gentle mixing for 2 hours at room temperature.
4. Add 0.5mL of IP Buffer, centrifuge for 2-3 minutes at 2500 × *g* and discard supernatant. Repeat this step several times.
5. To elute the immune complex, add 50µL of Elution Buffer and incubate for 5 minutes. Centrifuge tube for 1-3 minutes at 2500 × *g* and collect the supernatant. Repeat this step and combine the two supernatant fractions.

   Alternatively, wash the complex-bound resin with 0.5mL water, centrifuge for 2-3 minutes at 2500 × *g*, and discard supernatant. Add Electrophoresis Loading Buffer to the complex-bound resin and incubate for 5 minutes at 95°C. Centrifuge the resin mixture at 2500 × *g*, collect the supernatant and evaluate by SDS-PAGE.
6. Adjust eluate to physiological pH by adding ~10µL of the Neutralization Buffer per 100µL of eluate. The IP products may be used directly for SDS-PAGE, or the buffer may be exchanged by dialysis or desalting column to one that is compatible with the specific downstream application (see Related Thermo Scientific Products).

Troubleshooting

Problem	Possible Cause	Solution
Column flow is exceedingly slow (i.e., < 0.5mL/minute)	Outgassing of buffers or sample on the column, which causes blockage of resin pores with microscopic air bubbles	Degas buffers and remove air bubbles from column (see Additional Information section for suggested Tech Tip protocol)
Considerable antibody purified, but no specific antibody of interest detected	Antibody of interest was at very low concentration	Use serum-free medium for cell supernatant samples
		Affinity purify the antibody using the specific antigen coupled to an a affinity support such as Thermo Scientific AminoLink Plus Immobilization Kit (Product No. 44894)
Antibody of interest purified, but it is degraded (as determined by lack of function in downstream assay)	Antibody was sensitive to low-pH Elution Buffer	Try using Gentle Ag/Ab Elution Buffer (see Related Thermo Scientific Products)
	Downstream application was sensitive to neutralized Elution Buffer	Desalt or dialyze eluted sample into suitable buffer
No antibody detected in any elution fraction	Sample was devoid of antibody species or subclass that binds to Protein G	Refer to the Binding Characteristics Table for Protein G (see Additional Information section)

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. The UltraLink Biosupport is useful for medium pressure techniques such as FPLC.

Table 1. Characteristics of the UltraLink Biosupport.

Support pH Stability:	1-13
Average Particle Size:	50-80microns
Exclusion Limit:	> 2,000,000daltons
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:	100psi (6.9bar)*
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 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
- $1cm^3$ = 1mL of buffer
- cm^3/minute = Measured flow rate per minute (i.e., milliliter of effluent collected in 1 minute)

$$\text{Linear velocity/minute} = \frac{cm^3/\text{minute}}{\pi r^2} \quad \text{AND} \quad \text{Linear velocity/hour} = (\text{linear velocity/minute})(60\ \text{min/hr})$$

Therefore,

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

C. Information Available from our Website

- Tech Tip #34: Binding characteristics of Protein A, Protein G, Protein A/G and Protein L
- Tech Tip #4: Batch and spin cup methods for affinity purification of proteins
- Tech Tip #13: Pack beaded affinity resin into columns
- 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

Related Thermo Scientific Products

89868	**Pierce Centrifuge Columns, 0.8mL capacity,** 50/pkg
89896	**Pierce Centrifuge Columns, 2mL capacity,** 25/pkg
89897	**Pierce Centrifuge Columns, 5mL capacity,** 25/pkg
89898	**Pierce Centrifuge Columns, 10mL capacity,** 25/pkg
66382	**Slide-A-Lyzer® Dialysis Cassette Kit, 10K MWCO, 3mL**
69576	**Slide-A-Lyzer MINI Dialysis Device Kit, 10K MWCO, 0.1mL**
89893	**Zeba™ Spin Desalting Columns, 7K MWCO, 10mL**, 5/pkg
89882	**Zeba Spin Desalting Columns, 7K MWCO, 0.5mL**, 25/pkg
21027	**Gentle Ag/Ab Elution Buffer**, 500mL
44894	**AminoLink® Plus Immobilization Kit**

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

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