INSTRUCTIONS

Thermo SCIENTIFIC

# Protein A/G UltraLink® Resin

53132 53133 53135

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| Number | Description |
|---|---|
| **53132** | **Protein A/G UltraLink Resin,** 2mL settled resin |
| **53133** | **Protein A/G UltraLink Resin,** 10mL settled resin |
| | Support: Supplied as 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 of settled resin |
| **53135** | **Protein A/G Plus UltraLink® Resin**, 2mL settled resin |
| | Support: Supplied as 50% slurry (e.g., 2mL of settled resin is equivalent to 4mL of 50% slurry) containing 0.02% sodium azide |
| | Binding capacity: > 28mg human IgG/mL of settled resin |

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

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## Introduction

Immobilized Protein A/G is an excellent purification tool for most immunoglobulins. Protein A/G binds to all human IgG subclasses and also binds somewhat to IgA, IgE, IgM and, to a lesser extent, IgD; therefore, it has a broader binding range than either Protein A or Protein G individually. Unlike non-recombinant Protein G, Protein A/G does not bind serum albumin because the gene sequence coding for the albumin-binding site has been eliminated. Protein A/G is effective for mouse monoclonal antibody purification from IgG subclasses because Protein A/G binds all mouse IgG subclasses but does not bind murine IgA, IgM or serum albumin. In addition, the binding event is not as pH dependent as Protein A.

Protein A/G is a genetically engineered protein (MW ~50,500; apparent MW by SDS-PAGE ~40,000-45,000) that combines the IgG binding profiles of both Protein A and Protein G. The secreted Protein A/G contains four Fc-binding domains from Protein A and two from Protein G, making it a more universal tool to investigate and purify immunoglobulins.

The Thermo Scientific Protein A/G UltraLink Resins use an azlactone-activated support that is hydrophilic, charge-free, high capacity, highly crosslinked, rigid, copolymeric and porous. The physical properties of this support are important considerations when using samples requiring fast-flow techniques and large-scale applications. Agarose supports are useful for gravity-flow procedures; however, more rigid supports are required if flow rates require pressures greater than 25psi. The Thermo Scientific UltraLink Biosupport is useful for medium pressure techniques such as FPLC. More specific information regarding this support is detailed in the Additional Information Section.

## Important Product Information

- The flow rate of liquid through the column will be slow if gravity flow is used. For best results, attach the packed 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. For information and calculations for determining linear flow rate see the Additional Information Section.
- Our Binding and Elution Buffers 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. Therefore, optimization may be necessary when using other buffers.
- For optimal recovery, use a sample size such that the expected IgG load on the column is less than 80% of the binding capacity (see Product Description on page 1 for binding capacities). The total IgG content of serum is approximately 10-15mg/mL. The concentration of antibody in tissue culture supernatant varies considerably among hybridoma clones.
- Antibodies from fetal bovine serum (FBS) culture media supplement will be purified along with the antibody of interest

## Procedure for Antibody Purification Using a Gravity-flow Column

**Note:** The following protocol is for using a gravity-flow column packed with 2 ml of settled resin (i.e., 4mL of the 50% slurry). When using columns containing other resin bed volumes, reagent amounts must be adjusted accordingly.

**A. Additional Materials Required**

- Column capable of containing at least 2mL resin-bed volume such as the Product No. 89896 or 89897.
- Binding Buffer: Protein A/G IgG Binding Buffer (Product No. 54200)
- Elution Buffer: IgG Elution Buffer (Product No. 21004 and 21009) or 0.1M glycine, pH 2-3
- Neutralization Buffer: Prepare 1mL of high-ionic strength alkaline buffer such as 1M phosphate or 1M Tris, (pH 7.5-9)
- (Optional): Thermo Scientific Slide-A-Lyzer Dialysis Cassette or Zeba™ Spin Desalting Column (Product No. 89893) for buffer exchange

**B. Immunoglobulin Purification Procedure**

1. Equilibrate all components to room temperature before use.
2. Pack the column with 4mL of Protein A/G UltraLink Resin slurry.
3. Equilibrate column by adding 5mL of the Binding Buffer and allowing the solution to drain through.

   **Note:** To avoid air bubbles being drawn into the resin, remove the top cap before the bottom cap when opening column.
4. To maintain optimal ionic strength for binding, dilute sample at least 1:1 with Binding Buffer.

   **Note:** Plasma will 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 column.
5. Apply diluted sample to the column and allow it to flow completely into the resin. 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.
6. Wash the column with 10-15mL of Binding Buffer. If desired, verify that all non-bound proteins are removed from the column by collecting separate 2mL fractions 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 to 1mL of eluate. Monitor the elution by measuring the absorbance at 280nm or by protein assay such as Pierce BCA Protein Assay Kit (Product No. 23225).
8. Pool the eluted antibody fractions that have 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 8-15mL of Elution Buffer. Columns can be regenerated at least 10 times without significant loss of binding capacity.
10. For storage, wash column with 5mL of water containing 0.02% sodium azide. When approximately 3mL of solution remains above the top disc, replace the bottom cap followed by the top cap on the column. Store column upright at 4°C.

## Example Immunoprecipitation (IP) Procedure Using Protein A/G Resin

### A. Additional Materials Required

- 1.5-2mL microcentrifuge tube
- IP Buffer: 25mM Tris, 150mM NaCl; pH 7.2 (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 A/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 A/G Resin (100µL 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 A/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.

## Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| Flow of the column is exceedingly slow (i.e., < 0.5mL/minute) | Outgassing of buffers or sample on the column, which resulted in 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 low concentration | Use serum-free medium for cell supernatant samples |
| | | Affinity purify the antibody using the specific antigen coupled to an activated affinity support such as the 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 Thermo Scientific Gentle Ag/Ab Elution Buffer (Product No. 21027) |
| | Downstream application was sensitive to neutralized Elution Buffer | Desalt or dialyze eluted sample into suitable buffer |
| No protein detected in any elution fraction | Sample was devoid of antibody species or subclass that binds to Protein A/G | Refer to the Binding Characteristics Table for Protein A/G (See Additional Information) |

## 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:** | 50-80µm |
| **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 (6.9bar)* |
| **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)
- $\pi r^2$ = 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)

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

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

Therefore,

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

### 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

**20421** **Pierce Protein A/G Agarose,** 3mL

**20423** **Pierce Protein A/G Plus Agarose,** 2mL

**88802** **Pierce Protein A/G Magnetic Beads,** 1mL

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