INSTRUCTIONS

UltraLink Biosupport

53110 53111

0678.4

Number	Description
53110	**UltraLink Biosupport,** 1.25 g (~10 ml of resin)
53111	**UltraLink Biosupport,** 6.25 g (~50 ml of resin)

Swell Volume: 7.9-10 ml resin/g beads (see label for lot-specific swell volume)
Functionality: > 230 µmol/g = > 28.75 µmol/ml resin = > 0.23 mEq/g = > 0.029 mEq/ml resin

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

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Introduction

The Thermo Scientific UltraLink Biosupport is a pre-activated porous resin for use in affinity chromatography. These beads are composed of a bisacrylamide/azlactone copolymer that is slightly hydrophobic in its active form and highly crosslinked. The azlactone functionality is co-polymerized with the matrix material resulting in a high level of functionality throughout the porous bead.

The support is provided in dry form and reacts rapidly with nucleophiles via a ring-opening reaction to attach the amine-containing ligand through covalent linkages (Figure 1). The coupling reaction results in stable amide bonds with no leaving group or toxic chemical byproduct. The beads have an open architecture producing a high surface area and pore volume that allows coupling to a wide range of proteins and small molecules. The rigid polymeric property of this support provides excellent utility in medium-to-low pressure chromatography applications.

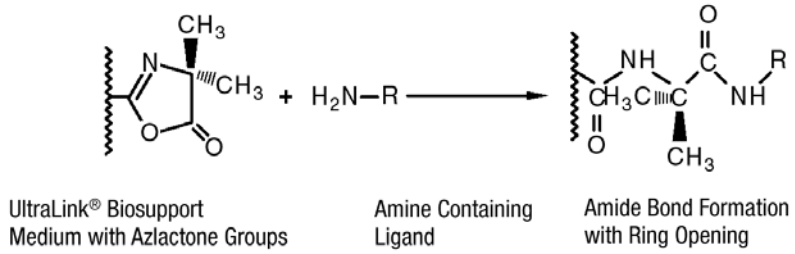

Figure 1. Reaction of azlactone ring on UltraLink Biosupport with amine-containing ligand.

Specific Physical Characteristics of the UltraLink Biosupport

The UltraLink Support is an azlactone-activated support that is hydrophilic, charge-free, high capacity, highly crosslinked, rigid, copolymeric and porous (see Table 1). The porosity, rigidity and durability of this support are important considerations when using large sample volumes requiring fast-flow techniques and large-scale applications.

Table 1. Characteristics of the UltraLink Biosupport.

Support pH Stability:	1-13
Temperature stability:	4-40°C
Average Particle Size:	50-80 microns
Exclusion Limit:	> 2,000,000 daltons
Average Surface Area:	> 250 m^2/g of beads
Average Pore Volume:	> 1 ml/g of beads
Pore Size:	1,000 Å
Maximum Pressure:	100 p.s.i. (6.9 bar)*
Maximum Linear Velocity:	3,000 cm/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.

Important Product Information

- Avoid coupling solutions containing primary amines (e.g., Tris or glycine) as they compete with the coupling reaction. Also avoid phenols, potassium thiocyanate, β-mercaptoethanol or ammonium sulfate as these chemicals may react with the azlactone functionality of the biosupport and cause a reduction in coupling efficiency.
- Any coupling buffer may be used provided it does not contain primary amines. Adjust the salt concentration and pH to suitable conditions for the protein being coupled.
- For most ligands, adding a lyotropic salt (e.g., 0.6 M sodium citrate or 0.8 M sodium sulfate) to the coupling solution will greatly increase the coupling efficiency (see Figure 7: Effects of Salts on Coupling).
- Quenching of excess azlactone groups after protein coupling is required because of the high level of functionality. The beads can be quenched using 3.0 M ethanolamine, pH 9.0 at room temperature for at least 2.5 hours. Other quench solutions may be used, including 1.0 M ethanolamine, pH 9.0; 0.1 M glucosamine in 1.0 M sodium sulfate, pH 8.5; and primary amine buffers such as 1.0 M Tris, pH 8.0; and alkylamines (e.g., butylamine or methylamine).
- Columns may be prepared for either gravity or flow procedures. Use 75% v/v bead slurry to prepare columns fitted with a < 25 µm frit. When preparing a column for operation at high flow rates (> 500 cm/hour), pack the beads at a flow rate 25% higher than the operational flow rate for a minimum of 10 column volumes.

Additional Materials Required

- Amine-containing ligand in appropriate buffer (see Important Product Information Section)
- Vortex Mixer
- Rocking platform or rotating device
- Quench Solution: 3.0 M ethanolamine, pH 9.0
- Centrifuge filtration device with < 25 µm filters or Pierce Spin Cups (Product No. 69702) for small volume samples
- Wash solutions, such as phosphate-buffered saline (e.g., BupH™ Phosphate Buffered Saline Packs, 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2; Product No. 28372) and 1.0 M sodium chloride (NaCl)

General Coupling Instructions

Note: The entire coupling reaction can be completed within 6 hours. Read all instructions before using this product.

1. Weigh sufficient beads for the column. Refer to the lot-specific swell volume to determine the amount of beads needed. Coupling may be performed in a centrifuge tube or a beaker.
2. Add the protein solution directly to the dry beads. Beads do not require hydration before use; the coupling buffer in the sample sufficiently hydrates the beads.

   Note: For most ligands, adding a lyotropic salt can greatly increase the coupling efficiency (see Figure 7).
3. Briefly vortex sample at medium speed to suspend beads. Gently rock or rotate sample for 1 hour at room temperature (RT). Coupling may also be performed at 4°C or 37°C. Do not use a magnetic stir bar as bead damage may result.
4. Centrifuge sample at 1,200 × *g* at RT for 5-10 minutes to pellet beads. Alternatively, filter sample using a 25 µm frit or, for small volumes, the Pierce Spin Cups may be used to separate the protein solution from the beads.
5. Remove supernatant by aspiration or decanting, being careful to retain beads in the tube. The supernatant may be used to determine the amount of ligand that did not couple to the beads.

   Note: Triton® X-100 surfactant is used in the production of the beads and may interfere with an A_{280} measurement of the uncoupled protein, therefore, use the Pierce BCA Protein Assay (Product No. 23225).
6. Add quench solution to the beads at 10 times bead volume to block non-reacted sites (see Important Product Information Section).
7. Vortex and gently rock or rotate sample for 2.5 hours.
8. Centrifuge sample at 1,200 × *g* for 5-10 minutes to pellet the beads. Remove and discard supernatant. Resuspend beads in 10 times the bead volume of PBS.
9. Vortex sample at medium speed to resuspend beads in the wash solution and gently rock or rotate sample for 15 minutes.
10. Centrifuge sample at 1,200 × *g* for 5-10 minutes to pellet the beads. Remove and discard supernatant.
11. Resuspend bead pellet in a high-salt wash solution, such as 1.0 M NaCl, to remove nonspecifically attached protein.
12. Vortex and gently rock or rotate sample for 15 minutes.
13. Centrifuge sample at 1,200 × *g* for 5-10 minutes to pellet the beads.
14. Resuspend beads with 10 times the bead volume of PBS. Vortex and gently rock or rotate sample for 15 minutes. Centrifuge sample at 1,200 × *g* for 5-10 minutes to pellet the beads. Remove and discard supernatant. Repeat this wash once.
15. Resuspend beads in a suitable buffer such as PBS. Store the ligand-coupled support at 4°C. If desired, a preservative solution may be included.

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 3,000 cm/hour.

The linear flow rate through a cylindrical column can be calculated if the height of the resin bed and the column's inside diameter is known, and if column effluent is collected and measured for a given time (see calculations below).

Calculations:

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

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

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

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

Additional Information and Product Data

Pressure-Flow Relationship

UltraLink Biosupport is ideal for low- and medium-pressure chromatography. The relationship between the differential pressure on the beads and the linear flow velocity of fluid through the column is illustrated in Figure 2.

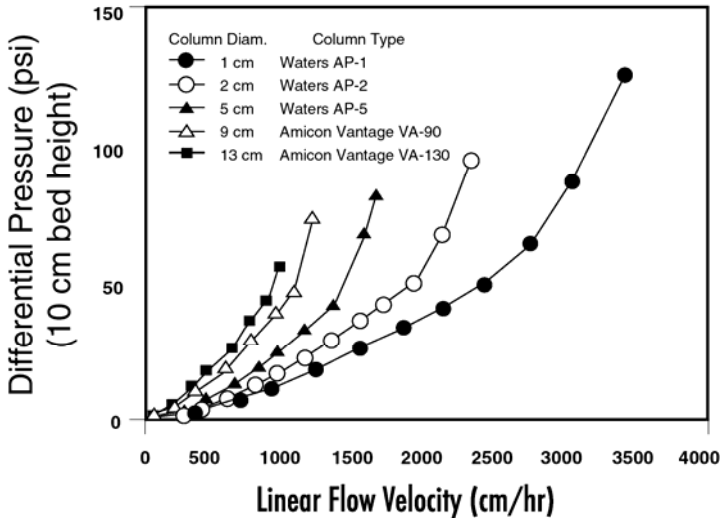

Figure 2. Pressure-flow relationship of UltraLink Biosupport.

Coupling of Proteins as a Function of pI and Molecular Weight

The UltraLink Biosupport can couple proteins of various isoelectric points (pI) and molecular weights effectively and efficiently (Table 2).

Table 2. Coupling of proteins as a function of pI and molecular weight.*

Protein	pI	Molecular Weight (kD)	Protein Offered (mg/ml)	% Coupling	Capacity (mg/ml)
Pepsin	2.9	34	1.60	46	8.6
Fetuin	3.3	49	0.76	87	7.9
BSA	4.9	66	1.82	68	14.3
Apoferritin	5.0-5.5	440	1.47	43	7.4
Human IgG	5.8-7.3	160	1.10	92	11.7
HRP	6.1-7.2	40	1.73	35	6.8
Carbonic anhydrase	6.2	29	1.67	75	14.9
Myoglobin	6.8-7.8	17	1.81	48	9.9
Ribonuclease	9.5	14	1.77	57	11.6
Lysozyme	11.0	14	1.59	56	10.3

*Proteins were prepared in 0.8 M sodium citrate, 0.1 M bicarbonate; pH 8.6 (human IgG was prepared in 0.5 M sodium citrate). Coupling was performed for 1 hour at RT using ~10.5 mg beads. Pierce BCA Protein Assay Reagent (Product No. 23225) was used to determine protein amount coupled to the beads.

Effect of Time and Temperature on Protein Coupling

Proteins couple rapidly and with high efficiency to UltraLink Biosupport at various temperatures (Figure 3).

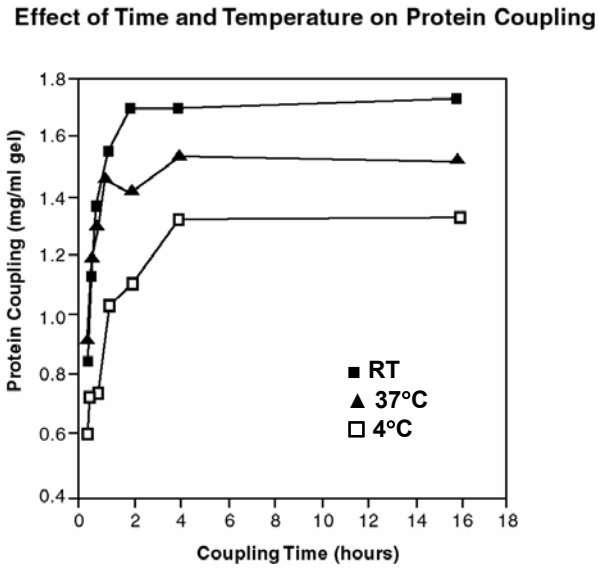

Figure 3. Effect of time and temperature on protein coupling to UltraLink Biosupport. Goat anti-rabbit antibody was coupled using 10 mg beads and 1 mg protein/ml in 0.6 M sodium citrate, 0.1 M MOPS; pH 7.5. Coupled ligand was radiometrically determined.

Effect of Protein Concentration

Proteins couple with high coupling capacity and efficiency (80%) to UltraLink Biosupport using a wide range of concentrations. For IgG, maximal coupling capacity was not reached at 11 mg IgG bound per ml of beads (resin) or 88 mg IgG bound per gram of dry beads (Figure 4).

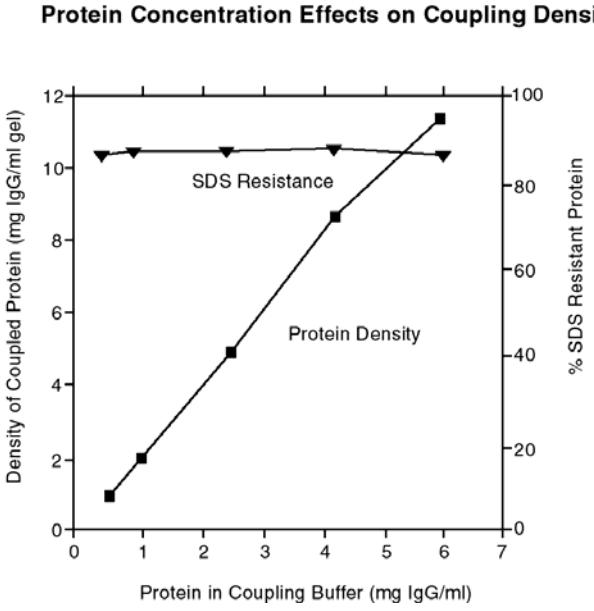

Figure 4. Effect of protein concentration on coupling to UltraLink Biosupport. Goat anti-rabbit IgG was prepared in 0.6 M sodium citrate, 0.1 M MOPS; pH 7.5 at various protein concentrations. The protein solution was added to the dry beads and coupled for 1 hour at RT. Beads were quenched with 3.0 M ethanolamine, pH 9.0 for 2.5 hours. To determine the protein amount coupled to the beads, protein-coupled beads were exposed to 1% sodium dodecyl sulfate (SDS) for 4 hours at 37°C. After incubation, beads were washed three times with 1% SDS for 15 minutes each wash. The amount of SDS resistant or covalently coupled protein was radiometrically determined.

Coupling Protein Solution Volume

Protein coupling can be accomplished efficiently in concentrated or dilute protein solutions and in varying volumes of coupling buffer (Figure 5). Using low or dilute protein solutions (e.g., 0.1 mg/ml) may result in a slight decrease in coupling efficiency with a 1 hour coupling time.

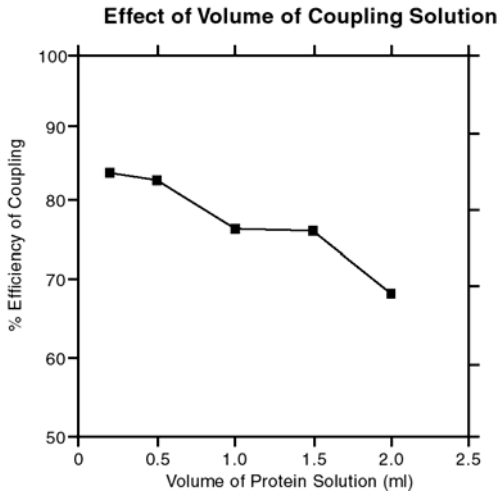

Figure 5. Coupling solution volume effects on efficiency of coupling to UltraLink Biosupport. To 10 mg of beads, 200 µg protein (goat anti-rabbit IgG) was added in varying amounts of coupling buffer (0.6 M sodium citrate, 0.1 M MOPS; pH 7.5).

Effect of Coupling Buffer pH on Ligand Binding

Proteins can be efficiently and effectively coupled to UltraLink Biosupport using a wide pH range. The most efficient coupling for goat anti-rabbit IgG was at a pH of 7.5 or greater (Figure 6).

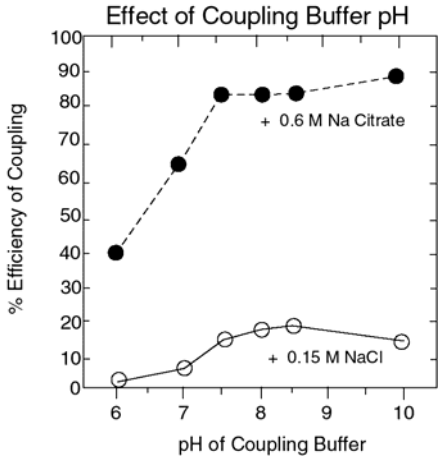

Figure 6. Effect of coupling buffer pH on ligand binding to UltraLink Biosupport. Goat anti-rabbit IgG (1 mg/ml) in coupling buffer was reacted with 10 mg of UltraLink Biosupport. Coupling buffers with 0.6 M sodium citrate or 0.15 M NaCl include 0.3 M MES at pH 6.0; 0.1 M phosphate at pH 7.0; 0.1 M MOPS at pH 7.5; 0.1 M TES at pH 8.0; 0.2 M Tricine at pH 8.5; 0.2 M carbonate at pH 10.0.

Effect of Salt Concentrations in the Coupling Buffer (Improved Coupling in the Presence of Lyotropic Salts)

Adding salts to the coupling buffer may enhance coupling efficiency, regardless of the pH. Goat anti-rabbit IgG was coupled to the beads in three different buffers. Three different salts were added to each buffer (sodium citrate, sodium sulfate or sodium chloride). The coupling efficiency increased as a function of increasing salt concentrations (Figure 7).

Note: Salt compatibility of the ligand must be considered.

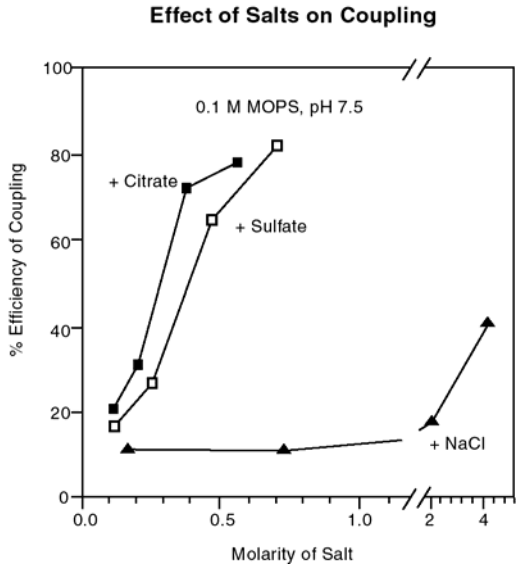

Figure 7. Effect of salt concentration in the Coupling Buffer for UltraLink Biosupport.

Reusability

Recombinant Protein A (rProtein A) was coupled to the beads and used to recover mouse IgG from tissue culture fluid. The specific capacity eluted from this column after greater than 130 cycles remained at 99% of the original capacity (Table 3).

Table 3. Elution capacity of UltraLink Biosupport after multiple uses.*

Cycle Number	IgG Eluted Capacity (mg/ml)	Area of Eluted Peak ($\times 10^{-6}$)	% Initial Capacity
1	5.47	1.43	100
20	5.66	1.45	116
50	5.26	1.42	96
100	5.28	1.43	97
139	5.41	1.37	99

*A 1.0 × 1.3 cm column was used with 1 ml of rProtein A coupled UltraLink Biosupport. The purification cycle was defined as: Load with 25 ml tissue culture fluid; ATCC HB-124 DB9G8 (mouse anti-insulin IgG_{2ak}) cultured in RPM1-1640 with 10% fetal calf serum (Gibco) using an Endotronics Acusyst-P5 hollow fiber reactor. The sample was filtered through a 0.45 µm Millipore Pelicon 6 cassette before applying to the column. Column was washed with PBS; pH 7.2, eluted with 0.1 M sodium citrate; pH 2.5, cleaned with 3.0 M guanidine•HCl in 20 mM phosphate; pH 7.2 and equilibrated with PBS; pH 7.2. The load phase of the cycle was run at 1 ml/minute (60 column volumes [CV]/hour) and all other phases were run at 3 ml/minute (180 CV hour).

Storage

Store the ligand-coupled support at 4°C. If desired, a preservative solution may be included. Storage in various solutions and subsequent binding was evaluated (Table 4).

Table 4. Effect of storage conditions on binding capacity of immobilized ligand on UltraLink Biosupport.*

Storage Solution (in PBS)	Specific Capacity with Storage at 4°C (mg/ml eluted IgG) at 1 month	Specific Capacity with Storage at 4°C (mg/ml eluted IgG) at 3 months
20% ethanol	18.5	21.6
0.05% sodium azide	20.0	19.1
0.01% benzyl alcohol	19.1	20.9
0.05% thimerosal	19.9	20.2
0.01 N sodium hydroxide	19.8	14.6

*rProtein A was coupled to UltraLink Biosupport. Before storage, the specific capacity of the coupled beads was determined using rabbit IgG and the beads allocated to the various storage conditions. Columns (1 ml) were poured and washed with 40 column volumes of PBS to remove the preservative solution before the 10 ml of 3 mg/ml rabbit IgG was loaded in 10 mM phosphate, pH 7.5. Specific capacities, using 0.1 M glycine, 2% acetic acid elution, pH 2.2, were determined by A_{280} measurements and chromatograms.

Note: Using 2% chlorhexidine gluconate in 20% ethanol as a preservative may cause precipitation during storage.

Cleaning Stability

UltraLink Biosupport coupled to proteins can be exposed to a variety of cleaning solutions without loss of specific eluted capacity. A stable protein (rProtein A) was coupled to the beads, and human IgG was applied to the columns. Specific capacities were determined (Table 5). Each column was exposed to five cycles as follows:

- Equilibration with PBS, pH 7.5
- IgG loading in PBS, pH 7.5 (10 ml at 3 mg/ml)
- Elution: 0.1 M glycine, 2% acetic acid, pH 2.2
- Cleaning solution for 10 column volumes
- Column equilibration with PBS, pH 7.5

Generally, high salt concentration and acidic and basic cleaning solutions have no effect on column capacities. Chaotropes can be successfully used at appropriate concentrations.

Table 5. Effect of cleaning solution on binding capacity of immobilized ligand on UltraLink Biosupport.

Cleaning Solution	Cycle 1 Capacity (mg IgG/ml resin)	Cycle 5 Capacity (mg IgG/ml resin)
4.0 M NaCl in PBS	19.9	19.9
4.0 M NaCl in 0.1 N HCl	18.2	18.9
6.0 M guanidine•HCl*	19.3	17.6
2.0 M Sodium thiocyanate	18.2	17.9
8.0 M urea	18.5	17.7
20% ethanol in PBS**	19.2	17.5
0.1 N sodium hydroxide	17.8	18.4
1.0 N ammonium hydroxide	18.6	20.2
0.1 N o-phosphoric acid	17.6	19.1

*Refer to the Reusability section for additional information on the effect of column cleaning with guanidine•HCl.

**Refer to Storage section for additional information on the use and effect of 20% ethanol in PBS.

Related Thermo Scientific Products

23225	**Pierce BCA Protein Assay**
69700	**Pierce Spin Cups – Paper Filter,** 600 µl capacity, 50 units and collection tubes
69702	**Pierce Spin Cups – Cellulose Acetate Filter,** 800 µl capacity, 50 units and collection tubes
89896	**Pierce Centrifuge Columns,** 2 ml resin-bed capacity, 25 each
89897	**Pierce Centrifuge Columns,** 5 ml resin-bed capacity, 25 each
89898	**Pierce Centrifuge Columns,** 10 ml resin-bed capacity, 25 each
21004	**IgG Elution Buffer,** 1 L
21009	**IgG Elution Buffer,** 3.75 L

Product References

Ju tongzhong, *et al.* (2002). Purification, characterization, and subunit sturcture of rat core 1 β1,3-galactosyltransferase, *J. Biol. Chem*. **277(1):**169-77.

Kornfeld, Rosalind, *et al*. (1998). Purification and multimeric structure of bovine N-acetylglucosamine-1-phosphodiester α-N-acetylglucosaminidase. *J. Biol. Chem.* **273(36),** 23203-23210.

General References

Coleman, P.L., *et al.* (1990). Immobilization of protein A at high density on azlactone-functional polymeric beads and their use in affinity chromatography. *J. Chromatogr.* **512:**345-63.

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

Coleman, P.L., *et al.* (1991). Effect of polyanionic salts on immobilization of protein A and antibody on azlactone-functional beads. *FASEB J.* **5**, A805 (#2528).

Hermanson, G.T., *et al.* (1992). Immobilized affinity ligand techniques, Academic Press: San Diego, California, U.S.A., pp. 28-30, 90-95,

Milbrath, D.S., *et al.* (1990). Azlactone-functional supports useful in affinity chromatography and other bioseparations. *AIChE Extended Abstracts*, #104E.

Rasmussen, J.K., *et al.* (1991/1992). Crosslinked, hydrophilic, azlactone-functional polymeric beads: A two-step approach. *Reactive Polymers* **16:**199-212.

Rasmussen, J.K., *et al.* (1992). Mechanistic studies in reverse-phase suspension copolymerization of vinyldimethylazlactone methylenebis (acrylamide). *Makromol. Chem., Macromol. Symp.* **54/55**, 535-550.

Rasmussen, J.K., *et al.* (1990). Hydrophilic, crosslinked, azlactone-functional beads - A new reactive support. *Polymer Reprints* **31(2):**442-3.