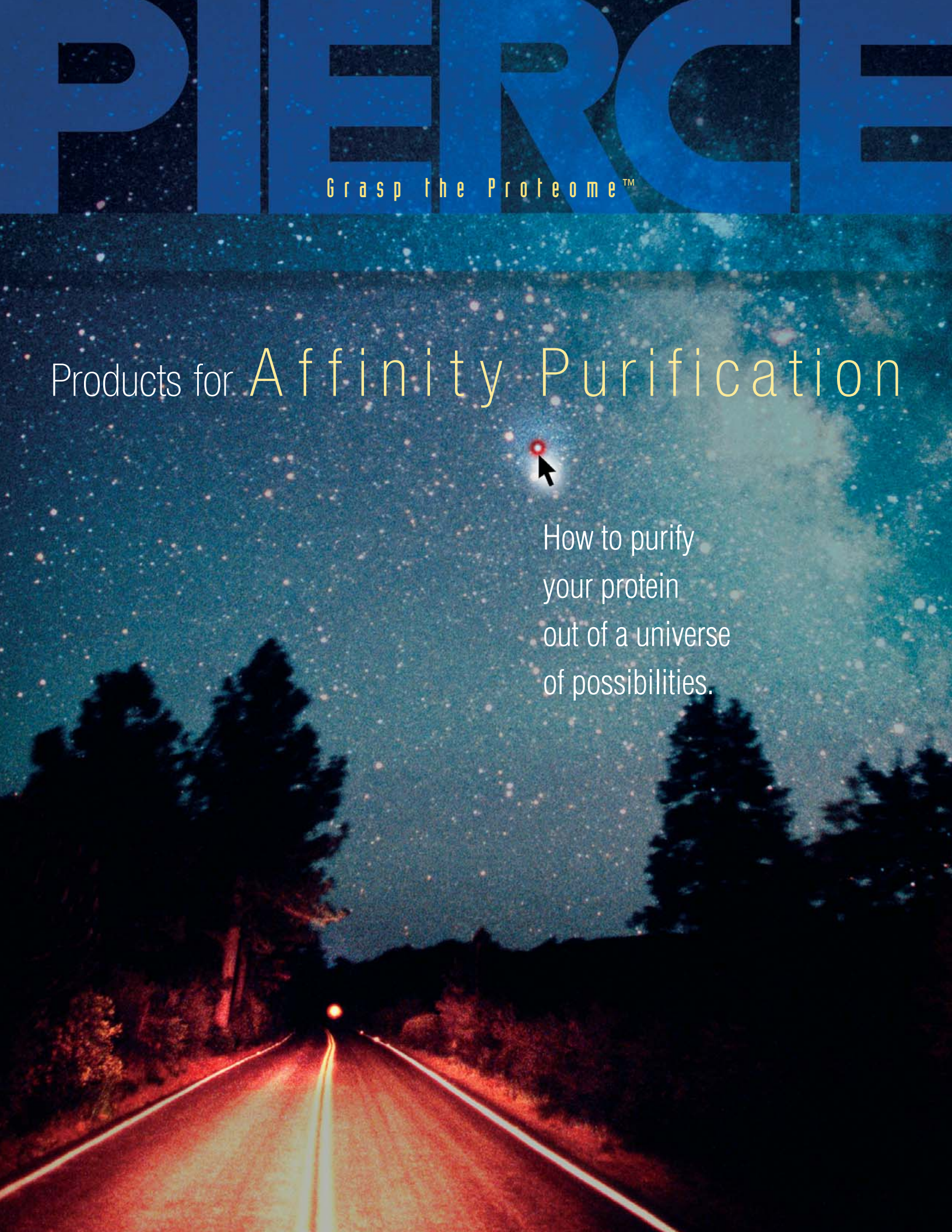
PIERCE

Grasp the Proteome™

Products for Affinity Purification



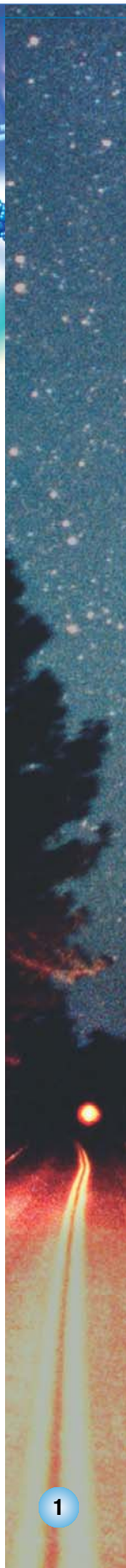
How to purify
your protein
out of a universe
of possibilities.

Contents

Introduction

Various methods are used to enrich or purify a protein of interest from other proteins and components in a crude cell lysate or other sample.

The most powerful of these methods is affinity purification, also called affinity chromatography, whereby the protein of interest is purified by virtue of its specific binding properties to an immobilized ligand. Pierce offers a number of immobilized protein or ligand products for affinity purification of antibodies, fusion-tagged proteins, biotinylated proteins and other proteins for which an affinity ligand is available.



Affinity purification makes use of specific binding that occurs between molecules and is used extensively for the isolation of biological molecules. A single pass through an affinity column can achieve a 1,000- to 10,000-fold purification of a ligand from a crude mixture. From a single affinity purification step, it is possible to isolate a compound in a form pure enough to obtain a single band upon SDS-PAGE analysis.

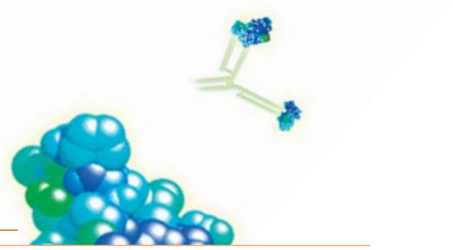
In affinity purification, a ligand is immobilized to a solid support. Once immobilized, it specifically binds its partner under mild buffer conditions (often physiological conditions such as phosphate buffered saline). After binding to the partner molecule, the support is washed with additional buffer to remove unbound components of the sample. An elution buffer is added, disrupting the interaction between the ligand and its binding partner by pH extremes (low or high), high salt, detergents, chaotrophic agents or the removal of some factor required for the pair to bind. Once released, the binding partner can be recovered from the support using additional elution buffer. The elution buffer can then be exchanged by dialysis or desalting into a more suitable buffer for storage or downstream analysis.

Activated affinity support products and kits enable a researcher to immobilize nearly any type of ligand to purify its binding partner(s). For example, if a peptide antigen is used to immunize animals and produce antibodies, the same peptide may be immobilized to a gel support and used to affinity-purify the specific antibody from animal serum. Alternatively, if a specific antibody is available against a particular protein of interest, it can be immobilized to a support and used to affinity-purify the protein from crude cell lysate. Purification with respect to nearly any binding interaction can be made by this approach.

Affinity purification products using either immobilized ligands or activated affinity support chemistries are available for use in several different formats. Most commonly, cross-linked beaded gel supports are used for gravity-flow column, spin-column or batch-scale purification procedures. Coated microplates are available for high-throughput screening applications, and magnetic particles are especially useful for affinity-based cell separation.

Proteins and other macromolecules of interest can be purified from crude extracts or other complex mixtures using a variety of methods. Precipitation is perhaps the simplest method for separating one type of macromolecule from another. For example, nucleic acids can be precipitated and thereby purified from undesired molecules in solution using ethanol and proteins can be selectively precipitated in the presence of ammonium sulfate.

Most purification methods involve some form of chromatography whereby molecules in solution (mobile phase) are separated based on differences in chemical or physical interaction with a stationary material (solid phase). Gel filtration (also called desalting, size-exclusion chromatography or SEC) uses a porous gel material to separate molecules based on size; large molecules are excluded from the internal spaces of the gel material while small molecules enter the resin pores, resulting in a longer path through the column. In ion-exchange chromatography, molecules are separated according to the strength of their overall ionic interaction with a solid-phase material. By manipulating buffer conditions, molecules of greater or lesser ionic character can be bound to or dissociated from the solid-phase material.



In contrast, affinity chromatography or affinity purification makes use of specific binding interactions between molecules. A particular ligand is chemically immobilized or "coupled" to a solid support so that when a complex mixture is passed over the column, only those molecules having specific binding affinity to the ligand are purified. Affinity purification generally involves the following steps:

1. Incubate crude sample with the immobilized ligand support material to allow the target molecule in the sample to bind to the immobilized ligand.
2. Wash away unbound sample components from solid support.
3. Elute (dissociate and recover) the target molecule from the immobilized ligand by altering the buffer conditions so that the binding interaction no longer occurs.

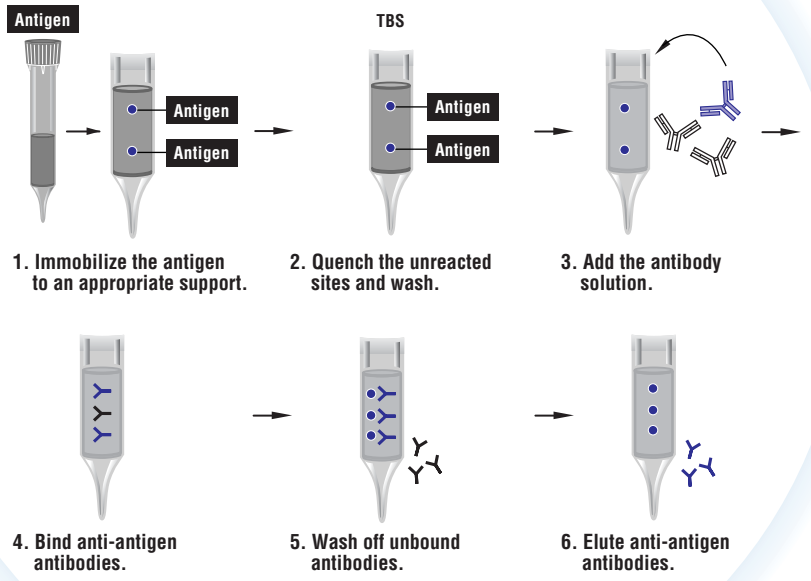
Ligands that bind to general classes of proteins (e.g., Protein A for antibodies) or commonly used fusion protein tags (e.g., glutathione for GST-tagged proteins) are available in pre-immobilized forms ready to use for affinity purification. Alternatively, more specialized ligands such as specific antibodies or antigens of interest can be immobilized using one of several activated affinity supports; for example, a peptide antigen may be immobilized to a support and used to purify antibodies that recognize the peptide.

Most commonly, ligands are immobilized or "coupled" directly to solid support material by formation of covalent chemical bonds between particular functional groups on the ligand (e.g., primary amines, sulfhydryls, carboxylic acids, aldehydes) and reactive groups on the support. However, other coupling approaches are also possible. In the GST Orientation Kit (Product # 78201), for example, a GST-tagged fusion protein is first bound to an immobilized glutathione support by affinity interaction with the GST tag and then chemically cross-linked to the support. The immobilized GST-tagged fusion protein can then be used to affinity-purify its binding partner(s). Likewise, Seize® X Immunoprecipitation Kits (e.g., Product # 45215) and IgG Orientation Kits (e.g., Product # 44990) involve binding and subsequent cross-linking of an antibody to immobilized Protein A or G.

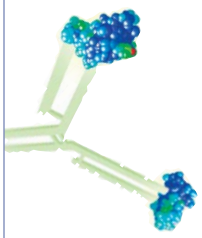
Historically, researchers have used affinity purification primarily to purify individual molecules of interest. Increasingly, proteomics research focuses on determination of disease states, cell differentiation, normal physiological functions and drug discovery involving interaction and expression of multiple molecules rather than individual targets. Consequently, the use of affinity methods has expanded to purification of native molecular complexes and forms the basis for co-immunoprecipitation (co-IP) and "pull-down" assays involving protein:protein interactions.

There are a variety of activated affinity supports that allow a researcher to purify proteins and other biological molecules of interest either alone or when present in complexes with their binding partners. Many of these supports will be discussed in the following pages.

Typical Antibody Purification Using an Immobilized Antigen Column



Binding and Elution Buffers for **Affinity Purification**



Most affinity purification procedures involving protein:ligand interactions use binding buffers, such as phosphate buffered saline (PBS), at physiologic pH and ionic strength. This is especially true when antibody:antigen or native protein:protein interactions are the basis for the affinity purification. Once the binding interaction occurs, the support is washed with additional buffer to remove unbound components of the sample. Nonspecific (e.g., simple ionic) binding interactions can be minimized by moderate adjustments to salt concentration or by adding low levels of detergent in the binding and/or wash buffer. Finally, elution buffer is added to break the binding interaction and release the target molecule, which is then collected in its purified form. Elution buffer can dissociate binding partners by extremes of pH (low or high), high salt (ionic strength), the use of detergents or chaotropic agents that denature one or both of the molecules, removal of a binding factor, or competition with a counter ligand. In most cases, subsequent dialysis or desalting is required to exchange the purified protein from elution buffer into a more suitable buffer for storage or downstream analysis. For more information on dialysis or desalting, order a free high-performance dialysis brochure from Pierce.

The most widely used elution buffer for affinity purification of proteins is 0.1 M glycine•HCl, pH 2.5-3.0. This buffer effectively dissociates most protein:protein and antibody:antigen binding interactions without permanently affecting protein structure. However, some antibodies and proteins are damaged by low pH, so eluted protein fraction(s) should be neutralized immediately by collecting the fractions in tubes containing 1/10th volume of alkaline buffer such as 1 M Tris•HCl, pH 8.5. Other elution buffers for affinity purification of proteins are listed in Table 1. In addition, Pierce offers several preformulated binding and elution buffers designed for affinity purification involving antibodies. See pages 4-5 and 40-41 for Pierce's line of pre-formulated binding and elution buffers.

Table 1. Common Possible Elution Systems for Protein Affinity Purification

Condition	Buffer
pH	100 mM glycine•HCl, pH 2.5-3.0
	100 mM citric acid, pH 3.0
	50-100 mM triethylamine or triethanolamine, pH 11.5
	150 mM ammonium hydroxide, pH 10.5
Ionic strength and/or chaotropic effects	3.5-4.0 M magnesium chloride, pH 7.0 in 10 mM Tris
	5 M lithium chloride in 10 mM phosphate buffer, pH 7.2
	2.5 M sodium iodide, pH 7.5
	0.2-3.0 sodium thiocyanate
Denaturing	2-6 M guanidine•HCl
	2-8 M urea
	1% deoxycholate
	1 % SDS
Organic	10% dioxane
	50% ethylene glycol, pH 8-11.5 (also chaotropic)
Competitor	>0.1 M counter ligand or analog

Binding and Elution Buffers for **Affinity Purification**

BupH™ Dry Buffers

The most advanced, versatile, time-saving buffer product line available.

The ultimate in convenience …

1. Reach for the sealed foil pack sitting conveniently on your bench top.
2. Open and add to deionized water.
3. The fresh buffer is ready to use in practical aliquots so there's no waste.

The ultimate in versatility …

- Routine buffers are designed for use in affinity purification and a variety of other applications.
- Using one buffer source maintains consistency and eliminates variables within the lab.
- Specialized buffers ideally support your work in specific chemistries and methods.

The ultimate in integrity …

- Unlike stored buffers, BupH™ Buffers are protected from contamination.
- Carry out applications with confidence in buffer quality.
- "Test-assured" with the Pierce commitment to world-class, quality management standards.

The ultimate in time savings …

- The making of specialized and routine buffers is no longer time-consuming.
- No component measurement, pH adjustment, quality validation, preparation tracking or refrigeration hassles.
- Move forward with your work by eliminating re-tests due to buffer problems.

BupH™ Borate Buffer Packs

Ideal for protein modification procedures that require an alkaline pH.

Each pack yields 50 mM borate, pH 8.5 after adding 500 ml of deionized water (20 liters total).

Ordering Information

Product #	Description	Pkg. Size
28384	BupH™ Borate Buffer Packs	40 pack

BupH™ Carbonate-Bicarbonate Buffer Packs

Ideal for microplate coating for RIA and EIA techniques.

Each pack yields 0.2 M carbonate-bicarbonate buffer, pH 9.4 when dissolved in 500 ml deionized water (20 liters total).

Ordering Information

Product #	Description	Pkg. Size
28382	BupH™ Carbonate-Bicarbonate Buffer Packs	40 pack

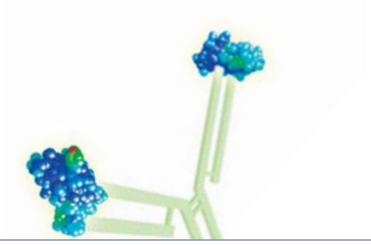
BupH™ Citrate-Carbonate Buffer Packs

Great for use with UltraLink® Supports.

Each pack yields 0.6 M sodium citrate, 0.1 M sodium carbonate, pH 9 when dissolved in 100 ml of deionized water (1 liter total).

Ordering Information

Product #	Description	Pkg. Size
28388	BupH™ Citrate-Carbonate Buffer Packs	10 pack



BupH™ Citrate-MOPS Buffer Packs

Great for use with UltraLink® Supports.

Each pack yields 0.6 M sodium citrate, 0.1 M MOPS buffer, pH 7.5 when dissolved in 100 ml of deionized water (1 liter total).

Ordering Information

Product #	Description	Pkg. Size
28386	BupH™ Citrate-MOPS Buffer Packs	10 pack

BupH™ MES Buffered Saline Packs

Ideal for use with carbodiimide-coupling chemistries.

BupH™ MES Buffered Saline Packs are designed for use with carbodiimide-coupling chemistries. They are designed for use with Immobilized DADPA (Product # 20266) and EDC. One pack of BupH™ MES Buffered Saline dissolved in 500 ml of water yields 0.1 M MES (2-[*N*-Morpholino]ethanesulfonic acid), 0.9% NaCl, pH 4.7.

Ordering Information

Product #	Description	Pkg. Size
28390	BupH™ MES Buffered Saline Packs	10 pack

BupH™ Modified Dulbecco's PBS Packs

A ready-to-use PBS for immunoassays.

Each pack yields 500 ml of 0.008 M sodium phosphate, 0.002 M potassium phosphate, 0.14 M sodium chloride and 0.01 M potassium chloride, pH 7.4 when dissolved in 500 ml deionized water (20 liters total).

Ordering Information

Product #	Description	Pkg. Size
28374	BupH™ Modified Dulbecco's PBS Packs	40 pack

BupH™ Phosphate Buffered Saline Packs

Ideal for cross-linking and biotinylation.

Each pack yields 500 ml of 0.1 M phosphate, 0.15 M NaCl, pH 7.2 when dissolved in 500 ml deionized water (20 liters total).

Ordering Information

Product #	Description	Pkg. Size
28372	BupH™ Phosphate Buffered Saline Packs	40 pack

BupH™ Tris Buffered Saline Packs

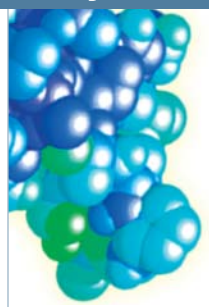
Ideal all-purpose binding buffer.

Each pack yields 500 ml of 25 mM Tris, 0.15 M NaCl, pH 7.2 when dissolved in 500 ml deionized water (10 pack makes 5 liters total; 40 pack makes 20 liters total).

Ordering Information

Product #	Description	Pkg. Size
28376	BupH™ Tris Buffered Saline Packs	40 pack
28379	BupH™ Tris Buffered Saline Packs	10 pack

Solid Supports for **Affinity Purification**



Affinity purification involves the separation of molecules in solution (mobile phase) based on differences in binding interaction with a ligand that is immobilized to a stationary material (solid phase). A support or matrix in affinity purification is any material to which a biospecific ligand may be covalently attached. Typically, the material to be used as an affinity matrix is insoluble in the system in which the target molecule is found. Usually, but not always, the insoluble matrix is a solid. Hundreds of substances have been described and employed as affinity matrices.

Useful affinity supports are those with a high surface area to volume ratio, chemical groups that are easily modified for covalent attachment of ligands, minimal nonspecific binding properties, good flow characteristics, and mechanical and chemical stability. When choosing an affinity support or matrix for any separation, the most important question to answer is whether a reliable commercial source exists for the desired matrix material in the quantities required. Fortunately, Pierce offers a wide range of practical and efficient matrices in volumes ranging from 1 ml to much larger bulk quantities.

Porous Gel Supports

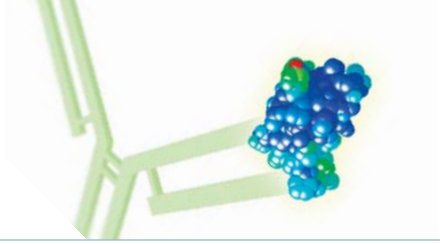
Porous gel supports generally provide the most useful properties for affinity purification of proteins. Pierce offers affinity purification products in two main porous gel support formats: cross-linked beaded agarose and UltraLink® Biosupport Medium. The various features of these two supports are listed in Table 2. Agarose is good for routine applications but crushes easily, making it suitable for gravity-flow column or small-scale batch procedures using low-speed centrifugation. UltraLink® Biosupport Medium is incompressible and can be utilized in high-pressure applications with a peristaltic pump or other liquid chromatography system. In addition, UltraLInk® Supports display extremely low nonspecific binding characteristics because they are polyacrylamide-based. Both supports perform well in typical gravity-flow column purification and immunoprecipitation procedures.

SwellGel® Discs

Pierce has developed a unique dehydrated and pelleted format for cross-linked beaded agarose gel called SwellGel® Discs. Immobilized ligands in the SwellGel® Disc format are especially suited for high-throughput purifications because the discs are easily distributed into 96-well filter plates or small spin columns for direct rehydration with the sample. When the sample is added, most SwellGel® Discs hydrate to approximately 100 µl volume within 90 seconds, automatically initiating the binding step. SwellGel® Nickel and Cobalt Chelated Discs (Product # 89827 and 89838, respectively) hydrate to 200 µl.

Magnetic Particles

When a matrix is required for affinity purification of cells within a population, Pierce MagnaBind™ Beads are recommended. Magnetic affinity separation is a convenient method for isolating antibodies, antigens, lectins, enzymes, nucleic acids and cells while retaining biological activity. Samples containing the molecule of interest are incubated with beads that are derivatized with an antibody or other binding partner. A rare earth magnet is used to pull the MagnaBind™ Beads out of solution and onto a surface. The buffer can be carefully removed, containing any non-bound molecules or cells.



MagnaBind™ Beads consist of a silanized surface over an iron oxide core (Table 3). The silanized surface has been derivatized to contain active groups, such as carboxylic acids or primary amines, or specific affinity molecules such as streptavidin or goat anti-mouse, anti-rabbit or anti-rat IgG. Due to the nature of the MagnaBind™ Beads, strong elution conditions are not recommended with these products. When using MagnaBind™ Beads to purify certain cells from a population, elution procedures are not required, as the beads automatically dissociate from the cells within 48 hours due to cell surface turnover. See page 62 for a complete listing of MagnaBind™ Supports.

Table 2. Physical Properties of Porous Gel Supports

Support	4% Agarose (cross-linked beaded agarose)	6% Agarose (cross-linked beaded agarose)	UltraLink® Biosupport Medium (co-polymer of cross-linked *bis*-acrylamide and azlactone)
Bead	45-165 µm	45-165 µm	50-80 µm
Exclusion Limit	20,000,000 daltons	4,000,000 daltons	2,000,000 daltons (1,000 Å pore size)
Durability	Crushes under pressure	Crushes under pressure	Sturdy (>100 psi, 6.9 bar)*
Types of Chromatography	Gravity flow only	Gravity flow only	FPLC® Systems, medium pressure, gravity flow
Coupling Capacity	Medium	Medium	High
pH range	3-11	3-11	1-13
Form	Preswollen	Preswollen	Dry or preswollen

** Note: The indicated maximum pressure of 100 psi refers to the maximum pressure drop across the gel bed 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 actually be measuring the pressure drop across the column. Typical system pressures are usually much higher due to pumping though small I.D. tubing, auto-samplers, detectors, etc. When packed into a 3 mm ID x 14 cm height glass column, these exclusive supports have been run to approximately 650 psi (system pressure) with no visual compression of the gel or adverse effects on chromatography. These columns can be run at linear flow rates or 85-3,000 cm/hour with excellent separation characteristics.*

Table 3. Characteristics of Underivatized MagnaBind™ Beads

Composition	Silanized iron oxide
Magnetization	25-35 EMU/g
Type of Magnetization	Superparamagnetic (no magnetic memory)
Surface Area	>100 m^2/g
Bead Size	1-4 µm diameter
Settling Rate	4% in 30 minutes
Effective Density	2.5 g/ml
Number of Beads	1 x 10^8 beads/mg
pH Stability	Aqueous solution, above pH 4.0
Concentration	5 mg/ml

Note: To establish a microbe-free preparation, MagnaBind™ Beads can be washed with antibiotic medium or γ-irradiated.

Microplates

Another type of matrix that is commonly used for immobilization of proteins is polystyrene microplates. Proteins passively adsorb to the polystyrene surface through hydrophobic interactions. Generally, this adsorption of proteins onto the polystyrene surface occurs best in carbonate/bicarbonate buffer at an alkaline pH (9.0-9.5). In addition, polystyrene surfaces can be derivatized with certain chemistries that will allow peptides and other nonprotein molecules to adhere to the surface in order to perform affinity assays in the wells of the plates.

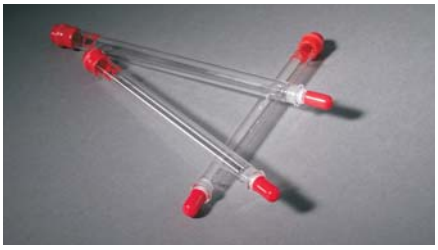
Pierce offers precoated plates to allow researchers an easy-to-use, consistent method for affinity purification or identification of specific molecules of interest. The plates offered include those specific for fusion proteins (6xHis, GST, GFP and MBP), antibodies (Protein A, Protein G, Protein A/G, Protein L, goat anti-mouse and goat anti-rabbit IgG), biotin (streptavidin and NeutrAvidin™ Biotin-Binding Protein) and those with reactive chemistries (maleic anhydride and maleimide) to allow binding of nonprotein samples that do not adsorb to the plastic microplate well surface.

Columns for **Affinity Purification**

Not only are several solid supports available, but these supports can be adapted for use in either large- or small-scale affinity purification procedures. For example, packed in a gravity-flow column, several milliliters of an agarose or UltraLink® Gel Support are generally useful for purifying 1-100 mg of target protein. However, 20-200 µl of gel can be used in a spin-cup or spin-column format with microcentrifuge tubes to purify 50-1,000 µg of target protein, or 10-50 µl of gel can be used batch-wise as a slurry directly in a microcentrifuge tube for even smaller-scale immunoprecipitation procedures. Columns and other Handee™ Sample Handling Devices, as well as purification kits using these devices, are available from Pierce.

Reusable Glass Columns

Unmatched convenience and versatility for chromatographic applications.



Highlights:

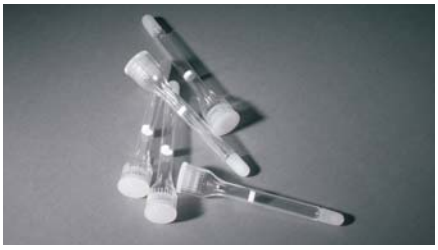
- Made of borosilicate glass; can be used with aqueous and organic eluents
- Supplied complete with polyethylene porous discs, end fittings and end cap

Ordering Information

Product #	Description	Pkg. Size
20055	**Reusable Glass Columns** Total Volume: 8 ml Includes: Polyethylene end fitting with 40 ± 10 µm pore size polyethylene hydrophilic disc and reusable end caps	6/pkg.

Disposable Plastic Columns

Automatic "stop-flow" action provided by porous polyethylene discs prevents column beds from drying out.



Highlights:

- Supplied complete with porous polyethylene discs, stoppers and end caps
- Compatible with most types of aqueous buffer eluents commonly used in chromatography
- Can be pre-packed and stored until needed

Ordering Information

Product #	Description	Pkg. Size
29920	**Disposable Polystyrene Columns** *Ideal for packing 0.5-2.0 ml bed volumes.*	100/pkg.
29922	**Disposable Polypropylene Columns** *Ideal for packing 1-5 ml bed volumes.*	100/pkg.
29924	**Disposable Polypropylene Columns** *Ideal for packing 2-10 ml bed volumes.*	100/pkg.
29923	**Disposable Polypropylene Funnels** *Buffer reservoirs that fit Product #'s 29920, 29922 and 29924.*	50/pkg.
29925	**Disposable Column Trial Pack** *Includes accessories plus two each of Product #'s 29920, 29922 and 29924 and one of Product # 29923.*	Trial Pack

Handee™ Devices for Fast and Flexible Separations

Helpful new tools for handling affinity resins.

Handee™ Devices are convenient tools in three formats for manipulation of small volumes of resin supports. Apply the resin and samples to one of the Handee™ Spin Cup Columns and use a microcentrifuge for washing and eluting sample. Once available only in our popular Seize® X Immunoprecipitation Kits, Spin Cup Columns are now sold separately.

The Handee™ Mini-Spin Columns include optional Luer-Lok® Adapters for performing washes and elutions with a syringe in addition to a microcentrifuge. There is no need to always use a microcentrifuge for separations! These easy-to-use Mini-Spin Columns can be used with our affinity supports.

Handee™ Resin Separators are ideal for use with larger volumes (250 µl-1 ml) of resin, without using a microcentrifuge. Simply slide the resin separator into a 16 mm x 100 mm test tube containing the support and sample. The separator compresses the resin away from the sample, which is then decanted out of the tube.

Ordering Information

Product #	Description	Pkg. Size
69700	Handee™ Spin Cup Columns	50 units
69705	Handee™ Mini-Spin Columns Plus Accessories *Does not include microcentrifuge tubes.*	25 units
69710	Handee™ Resin Separators	25 units
69715	Handee™ Microcentrifuge Tubes, 1.5 ml	72 tubes

Handee™ Spin Cup Columns
Chromatography Resin
Volume: 50-200 µl

Sample Volumes: 50-500 µl

Highlights:
- Quick separations using a microcentrifuge

Handee™ Mini-Spin Columns
Chromatography Resin
Volume: 20-400 µl

Sample Volumes: 50-800 µl

Highlights:
- Quick separations using a microcentrifuge or Luer-Lok® Adapter for use with a syringe
- Autoclavable at 120°C

Handee™ Resin Separators
Chromatography Resin
Volume: 250 µl-1ml

Sample Volumes: 1-10 ml

Highlights:
- No need to use a microcentrifuge or a syringe
- Requires a 16 mm x 100 mm test tube

Economy Mini-Spin Columns

Economy Mini-Spin Columns are empty, disposable microcentrifuge filter spin columns with a capacity of 0.8 ml. The columns can be packed with various chromatography media for rapid batch purification. Column material is polypropylene with a 25 µm polyethylene frit and twist-off tip. The columns fit into standard microcentrifuge tubes for use in a fixed-angle rotor microcentrifuge. Economy Mini-Spin Columns with screw caps and twist-off tips are an economical inexpensive alternative for procedures requiring empty, disposable microfilter spin columns.

Ordering Information

Product #	Description	Pkg. Size
89868	Economy Mini-Spin Columns, 0.8 ml capacity	Kit
	Includes: Mini-Spin Columns	50 each
	Screw Caps	50 each
89869	Economy Mini-Spin Columns, 0.8 ml capacity	Kit
	Includes: Mini-Spin Columns	4 x 50 each
	Screw Caps	4 x 50 each

Covalent Coupling of Affinity Ligands to Chromatography Supports

Affinity chromatography uses the specific interactions between two molecules for the purification of a target molecule. In practice, a ligand having affinity for a target molecule is covalently attached to an insoluble support and functions as bait for capturing the target from complex solutions. The affinity ligand can be virtually any molecule that can specifically bind the target without displaying significant nonspecific binding toward other molecules in the solution. Ligands that have been used for affinity separations include small organic compounds that are able to dock into binding sites on proteins, inorganic metals that form coordination complexes with certain amino acids in proteins, hydrophobic molecules that can bind nonpolar pockets in biomolecules, proteins with specific binding regions that are able to interact with other proteins, and antibodies, which can be designed to target any biomolecule through their antigen-binding sites.

The concept of using immobilized affinity ligands to target biomolecules has extended beyond chromatographic applications. Affinity ligands are now coupled to latex beads, nanoparticles, macro-beads, membranes, microplates, array surfaces, dipsticks and a host of other devices that facilitate the capture of specific biomolecules. The application of affinity targeting includes purification, scavenging (or removal of contaminants), catalysis (or modification of target molecules) and a broad range of analytical uses to quantify a target molecule in a sample solution.

Designing custom affinity supports that are able to target unique biomolecules requires methods to covalently link a ligand to an insoluble matrix. Regardless of the intended application, the chemical reactions that make ligand attachment possible are well characterized and facilitate the attachment of biomolecules through their common chemical groups. The types of functionalities generally used for attachment include easily reactive components such as primary amines, sulfhydryls, aldehydes, carboxylic acids, hydroxyls, phenolic groups and histidinyl residues. Usually, the solid-phase matrix first is activated with a compound that is reactive toward one or more of these functional groups. The activated complex then can form a covalent linkage between the ligand and the support, resulting in ligand immobilization.

The type of linkage that is formed between the matrix and the immobilized ligand affects the performance of the affinity support in a number of ways. A linkage that allows the coupled ligand to leach from the matrix will result in contamination of the purified protein and shorten the useful life of the affinity support. A linkage that introduces a charged functional group into the support can cause nonspecific binding by promoting ion-exchange effects. A linkage that alters the structure of the matrix can change the flow and binding characteristics of the support. Cyanogen bromide (CNBr)-activated supports are informative as an example of these principles. This popular immobilization method results in a linkage that:

1) Has a constant leakage of ligand from the matrix that becomes a contaminant in the purified preparation

2) Includes a charged isourea group in the linkage, resulting in nonspecific binding

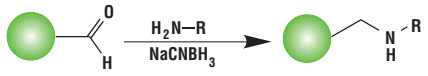
3) Causes extensive cross-linking of the matrix, reducing the ability of large molecules to penetrate into the interior of the resin

Pierce offers a number of activated affinity supports that are designed to couple ligands of every type via stable, uncharged covalent linkages that avoid introducing undesirable properties into the supports. The activation chemistry and protocols have been optimized to assure excellent coupling yields with minimal effort under a variety of conditions. Each activated support comes with instructions for use and literature references as examples. The associated kits contain all the coupling buffers, wash buffers and columns necessary to perform the ligand immobilization and produce a support ready to perform an affinity separation.

Coupling Affinity Ligand through Amine Groups

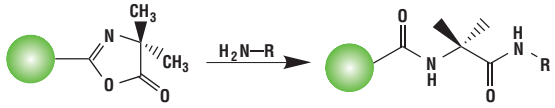
The most common functional target for immobilizing protein molecules is the amine group, which is present on the vast majority of proteins due to the abundance of lysine side chain ε-amines and N-terminal α-amines. Pierce AminoLink® Coupling Gel and AminoLink® Plus Coupling Gel are prepared from cross-linked agarose supports, and they are designed to create a stable linkage between amine groups and the support material. AminoLink® Gels are activated to contain numerous aldehyde groups, which can be used to immobilize amine-containing ligands by reductive amination.

The immobilization reaction using reductive amination involves the formation of an initial Schiff base between the aldehyde and amine groups, which then is reduced to a secondary amine by the addition of sodium cyanoborohydride. The cyanoborohydride reducing agent used during the coupling process is mild enough not to cleave disulfides in most proteins, and it will not reduce the aldehyde reactants – only the Schiff base intermediates. It is best to avoid stronger reducing agents such as sodium borohydride because of the potential for disulfide reduction of the protein and reduction of the aldehydes on the support to hydroxyls, effectively quenching the reaction. Depending on the type and amount of ligand present, a coupling reaction using reductive amination can achieve immobilization yields of greater than 85%.



Aldehyde Group on AminoLink® Coupling Gel — **Affinity Ligand Coupled via Secondary Amine Bond**

Another amine-reactive strategy that can be used for immobilization is the azlactone ring present in UltraLink® Biosupport Medium. A primary amine will react with an azlactone group in a ring-opening process that produces an amide bond at the end of a five-atom spacer. The group is spontaneously reactive with amines, requiring no additives or catalysts to drive the coupling process. The UltraLink® Biosupport Medium is supplied dry to assure stability of the azlactone groups prior to use. Adding a quantity of the support to a sample containing a protein or another amine-containing molecule causes immobilization to occur within about one hour. For protein immobilization at high yield, it is recommended that the coupling buffer contain a lyotropic salt, which functions to drive the protein molecules toward the bead surface. This brings the hydrophilic amines close enough to the azlactone rings to react quickly. The simple nature of coupling affinity ligands to the UltraLink® Biosupport Medium along with its inherently low nonspecific binding makes it one of the best choices for immobilization.

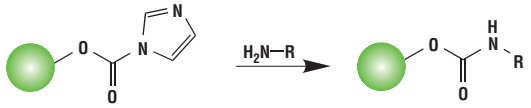


Azlactone Group on UltraLink® Biosupport Medium — **Affinity Ligand Coupled via Amide Bond**

Table 4. UltraLink® Binding Capacity for Various Proteins

Capacity	Protein	Coupling Buffer
35.0 mg/ml	Myoglobin	0.1 M CHES, 1.0 M sodium citrate, pH 9.0
21.5 mg/ml	Penicillin Acylase	0.1 M sodium phosphate, 1.1 M sodium sulfate, pH 7.4
20.9 mg/ml	α-chymotrypsin	0.1 M borate, 1.5 sodium sulfate, pH 9.0
35.5 mg/ml	BSA	0.1 M borate, 1.5 sodium sulfate, pH 9.0
29.8 mg/ml	Lysozyme	0.1 M borate, 1.0 sodium sulfate, pH 9.0
21.0 mg/ml	Human IgG	0.1 M borate, 1.5 sodium sulfate, pH 9.0

A third option for immobilizing amine-containing affinity ligands is the use of carbonyl diimidazole (CDI) to activate hydroxyls on agarose supports to form reactive imidazole carbamates. This reactive group is formed on the support in organic solvent and stored as a suspension in acetone to prevent hydrolysis. Reaction of the support in an aqueous coupling buffer with primary amine-containing ligands causes loss of the imidazole groups and formation of carbamate linkages. The coupling process occurs at basic pH (8.5-10), but it is a slower reaction with proteins than reductive amination or azlactone coupling. Pierce Reacti-Gel® Supports are available with the CDI-activated group, and they are particularly adept at immobilizing peptides and small organic molecules. The reaction also can be done in organic solvent to permit coupling of water-insoluble ligands.



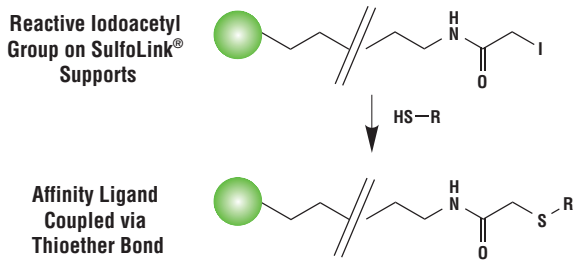
Reactive Imidazole Carbamate on Reacti-Gel® CDI Supports — **Affinity Ligand Coupled via Carbamate Bond**

Covalent Coupling of Affinity Ligands to Chromatography Supports

Coupling Affinity Ligands through Sulfhydryl Groups

It is often advantageous to immobilize affinity ligands through functional groups other than just amines. In particular, the thiol group can be used to direct coupling reactions away from active centers or binding sites on certain protein molecules. Since amines occur at many positions on a protein's surface, it is usually difficult to predict where a coupling reaction will occur. However, if sulfhydryl groups that typically are present in fewer numbers are targeted for immobilization, then coupling may be done at discrete sites in a protein or peptide. Thiol groups (sulfhydryls) can be indigenous within a protein molecule or they may be added through the reduction of disulfides or through the use of various thiolation reagents. Sulfhydryls also can be added to peptide affinity ligands at the time of peptide synthesis by adding a cysteine residue at one end of the molecule. This ensures that every peptide molecule will be oriented on the support in the same way after immobilization.

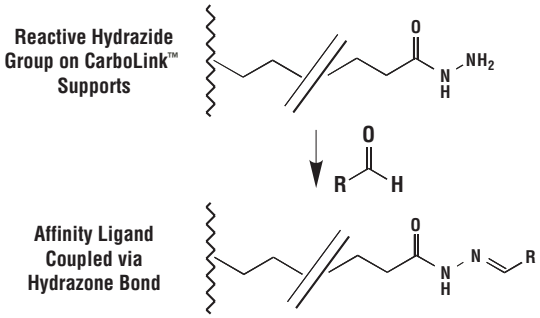
Pierce SulfoLink® Coupling Gel is designed to efficiently react with thiol-containing molecules and immobilize them through a thioether linkage. The support contains an iodoacetyl group at the end of a long spacer arm, which reacts with sulfhydryls through displacement of the iodine. Optimal conditions for the reaction are an aqueous environment at slightly basic pH, wherein amines are not very reactive toward the iodoacetyl function, but thiols are highly reactive due to their increased nucleophilicity. The thioether bond that is formed provides a stable linkage to any sulfhydryl-containing molecule.



Coupling Affinity Ligands through Carbonyl Groups

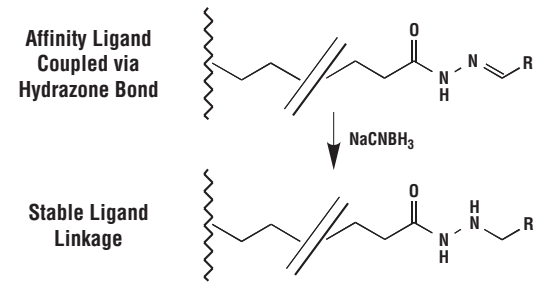
Most biological molecules do not contain carbonyl ketones or aldehydes in their native state. However, it might be useful to create such groups on proteins to form a site for immobilization that directs covalent coupling away from active centers or binding sites. Glycoconjugates, such as glycoproteins or glycolipids, usually contain sugar residues that have hydroxyls on adjacent carbon atoms, which can be periodate-oxidized to create aldehydes. Controlled oxidation using 1 mM sodium *meta*-periodate at 0°C will selectively oxidize sialic acid groups to form an aldehyde functionality on each sugar. Using higher concentrations of periodate (10 mM) at room temperature will result in oxidation of other sugar diols to create additional formyl groups. Aldehydes on the carbohydrate portion of glycoconjugates may be used to covalently link with affinity supports through an immobilized hydrazide, hydrazine or amine group by Schiff base formation or reductive amination.

Pierce CarboLink™ Coupling Gel contains long spacer arms that terminate in hydrazide groups. Reaction of the hydrazides with aldehydes forms hydrazone linkages, which are a form of Schiff base displaying better stability than those formed between an amine and an aldehyde. The CarboLink™ Gel can be used to immobilize glycoproteins, such as antibodies, after periodate oxidation of the carbohydrate. Coupling antibodies in this manner specifically targets the heavy chains in the Fc portion of the molecule. Since this is away from the antigen-binding sites at the end of the Fv regions, immobilization using this route often results in the best retention of antigen-binding activity.



The CarboLink™ Gel also may be used to couple carbohydrates and sugars through their reducing ends. Aldehyde- or ketone-containing sugars will react with the immobilized hydrazide groups without oxidation of other sugar hydroxyls. However, this reaction may be dramatically slower than coupling with oxidized sugars because these native aldehydes or ketones are usually tied up in acetal or ketal ring structures. These rings can open in aqueous solution to reveal the aldehyde or ketone, but the open structure is present only a small percentage of the time. Thus, the reducing ends of sugars have decreased reactivity toward an immobilized hydrazide, sometimes requiring days of reaction time to obtain acceptable immobilization yields.

Although the hydrazone bond created between the immobilized hydrazide and an aldehyde is much more stable than amine-aldehyde Schiff bases, to obtain a leach-resistant linkage it is recommended that the Schiff base be reduced with sodium cyanoborohydride. This is especially true if a ligand is coupled that has only a single point of attachment to the support. In other words, use the reduction protocol if the ligand contains only one aldehyde (or ketone) to couple with the immobilized hydrazide. Reduction of the hydrazone in this case will create a stable bond that will perform well in affinity chromatography applications.

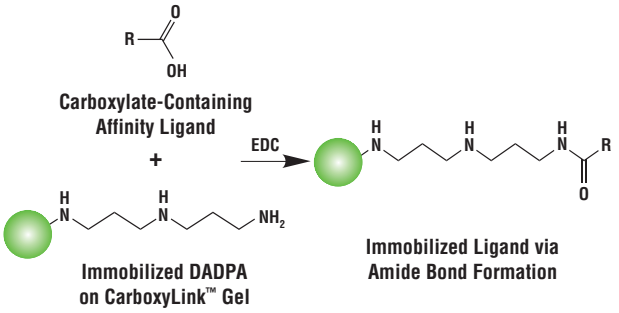


Coupling Affinity Ligands through Carboxyl Groups

The carboxyl group is a frequent constituent of many biological molecules. Particularly, proteins and peptides typically contain numerous carboxylic acids due to the presence of glutamic acid, aspartic acid and the C-terminal α-carboxylate group. Carboxylic acids may be used to immobilize biological molecules through the use of a carbodiimide-mediated reaction. Although no activated support contains a reactive group that is spontaneously reactive with carboxylates, chromatography supports containing amines (or hydrazides) may be used to form amide bonds with carboxylates. Molecules containing carboxylates may be activated to react with an immobilized amine (or hydrazide) through reaction with the water-soluble carbodiimide EDC.

EDC reacts with carboxylates to form an intermediate ester that is reactive with nucleophiles such as primary amines. The reaction takes place efficiently between about pH 4.5 and pH 7.5, and it is complete within two to four hours, depending on the temperature. The intermediate ester is subject to hydrolysis; therefore, it is beneficial if the amine-containing ligand to be immobilized is included in the reaction medium upon addition of EDC, so it can react immediately with the ester as it forms.

CarboxyLink™ Coupling Gel from Pierce or the UltraLink® DADPA Gel may be used to immobilize carboxylate-containing ligands by EDC. CarboxyLink™ Gel contains a nine atom spacer arm and UltraLink® DADPA contains a 12-atom spacer arm to minimize steric hindrance.



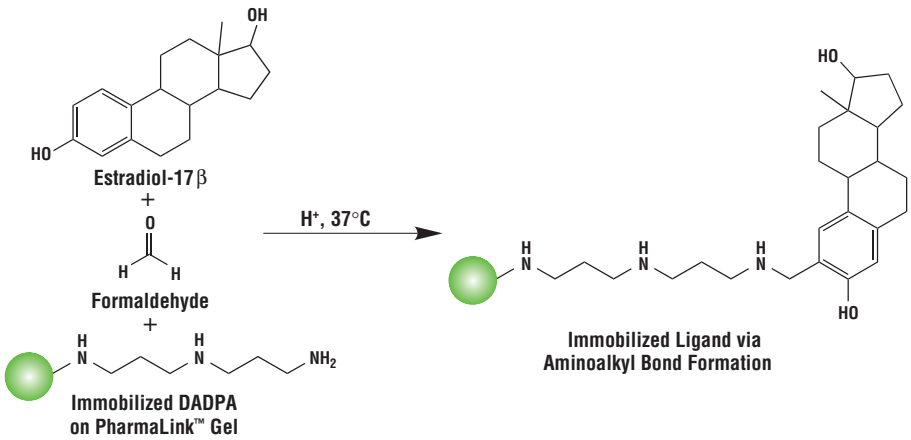
Coupling Affinity Ligands through Reactive Hydrogens

For molecules containing no easily reactive functional groups, immobilization may be difficult or even impossible using current technologies. Certain drugs, steroids, dyes and other organic molecules often have structures that contain no available "handles" for convenient immobilization. In other cases, functional groups that may be present on a molecule have low reactivity or are sterically hindered, prohibiting efficient coupling. Often, these compounds that are difficult to immobilize will have certain active (or replaceable) hydrogens that can be condensed with formaldehyde and an amine in the Mannich reaction. Certain hydrogens in ketones, esters, phenols, acetylenes, α-picolines, quinaldines and other compounds can be aminoalkylated using this reaction.

Formally, the Mannich reaction consists of the condensation of formaldehyde (or another aldehyde) with ammonia and another compound containing an active hydrogen. Instead of using ammonia, this reaction can be done with primary or secondary amines or even with amides.

Use of the Mannich reaction for the preparation of affinity supports offers some unique advantages beyond that of its effective use to immobilize compounds that are difficult to couple. For instance, polymerization is often a problem when using the Mannich reaction for solution-phase chemistries, especially when multiple reactive hydrogens are present on a molecule. When one of the reactive species is immobilized, the reaction is more controlled and undesirable side reactions are inhibited. Compounds with phenolic residues (often found in drugs) can be coupled without difficulty. In addition, the Mannich reaction is a superior alternative to the seldom-used diazonium coupling method. Both the diazonium group and the resultant diazo linkage are unstable. In contrast, immobilization using Mannich condensations result in very stable covalent bonds suitable for the most critical affinity separations.

Pierce has developed the PharmaLink™ Immobilization Kit, which is based on the principles of the Mannich reaction. The PharmaLink™ Gel included in the kit is immobilized diaminodipropylamine (DADPA), which is the source of the primary amine for the Mannich reaction. The kits also include coupling buffer, coupling reagent, wash buffer and accessories.



AminoLink® Plus Immobilization Kits and Coupling Gel

Offer an enhanced coupling protocol and an agarose matrix with greater rigidity and faster linear flow potential.

Highlights:

- Uses the basic technology of the original AminoLink® Coupling Gel with additional benefits including higher coupling capacities, better immobilization efficiencies and process-scale separation capabilities
- Gives enhanced coupling yields at neutral pH – coupling yields are even higher at a higher pH
- Faster flow rates on a more rigid support
- Successfully couples ligands over a wide range of concentrations, making it ideal for small amounts of ligand
- Support can be used a minimum of 10 times with no significant loss of capacity
- Immobilize 1-25 mg protein or 0.1-2 mg peptide/ml resin

Table 5. Immobilization of Various Proteins on AminoLink® Plus Using pH 7.2 Coupling Buffer

Protein	Protein Applied (mg/ml)	Protein Coupled (mg/ml)	Percent Coupled
Protein G	4.6	4.0	83
Mouse IgG	4.7	4.5	96
Rat IgG	4.7	4.4	93
GAM-IgG*	0.9	0.8	84
Human IgG	4.8	4.6	97
Human IgM	0.9	0.8	93

**GAM-IgG is goat anti-mouse IgG*

References
Beall, A., *et al.* (1999). *J. Biol. Chem.* **274(16)**, 11344-11351.
Nakasato, Y.R., *et al.* (1999). *Clin. Chem.* **45**, 2150-2157.
Allan, B.B., *et al.* (2000). *Science* **289**, 444-448.
Lu, R., *et al.* (2000). *J. Neurochem.* **74**, 320-326.

Ordering Information

Product #	Description	Pkg. Size
44894	**AminoLink® Plus Immobilization Kit**	Kit
	Includes: AminoLink® Plus Coupling Gel Columns	5 x 2 ml
	Neutral pH Coupling Buffer (pH 7.2)	500 ml
	Enhanced Coupling Buffer (pH 10)	500 ml
	Quenching Buffer	60 ml
	Wash Solution	240 ml
	Sodium Cyanoborohydride Solution	0.5 ml
	Accessories	
20394	**AminoLink® Plus Immobilization Trial Kit**	Trial Kit
	Includes: AminoLink® Plus Column Reagents and Buffers	1 x 2 ml
20501	**AminoLink® Plus Coupling Gel**	10 ml

AminoLink® Immobilization Kits and AminoLink® Coupling Gel

Links with primary amines (lysine residues and N*-terminus) on proteins, peptides, antigens or antibodies.*

Highlights:

- Ideal for coupling small amounts of protein with good recovery of biological activity
- Stable covalent bond in the form of a secondary amine between the gel and the protein
- Covalently couples with high efficiency over a wide range of pH and buffer conditions
- Low levels of ligand leakage
- Produces reusable immunoaffinity matrices
- Low nonspecific binding
- 4% cross-linked beaded agarose support, activated to form aldehyde functional groups
- Immobilize 1-20 mg protein or 0.1-2 mg peptide/ml resin

Table 6. AminoLink® Gel Coupling Efficiency vs. pH

pH	Coupling Efficiency of 9.58 mg Human IgG
4	91.8%
5	92.7%
6	89.1%
7	87.3%
8	85.3%
9	94.9%
10	98.4%

9.58 mg of Human IgG was coupled at varying pH levels to AminoLink® Gel according to the standard protocol.

References
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Assad, F.F., *et al.* (2001). *J. Cell Biol.* **152**, 531-543.
Zuk, P.A. and Elferink, L.A. (2000). *J. Biol. Chem.* **275(35)**, 26754-26764.

Ordering Information

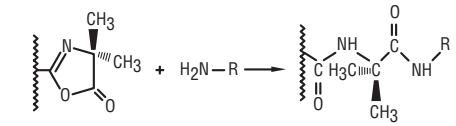
Product #	Description	Pkg. Size
44890	**AminoLink® Immobilization Kit**	Kit
	Includes: AminoLink® AffinityPak™ Columns	5 x 2 ml
	AminoLink® Coupling Buffer	240 ml
	AminoLink® Quenching Buffer	60 ml
	AminoLink® Wash Buffer	240 ml
	AminoLink® Reductant Accessories	190 mg
20384	**AminoLink® Immobilization Trial Kit**	Trial Kit
	Includes: AminoLink® AffinityPak™ Column Reagents and Buffers	1 x 2 ml
20381	**AminoLink® Coupling Gel**	10 ml
20382	**AminoLink® Coupling Gel**	50 ml
44892	**AminoLink® Reductant** (Sodium cyanoborohydride)	2 x 1 g

UltraLink® Biosupport Medium and Kit

For the fastest, most efficient coupling of proteins and small molecules.

Highlights:

- Pre-activated affinity chromatography support comprised of a hydrophilic, cross-linked *bis*-acrylamide/azlactone copolymer
- Low nonspecific binding[1]
- Covalently couples via amino, thiol and hydroxyl (including phenolic) functional groups with no residual byproducts – primarily immobilizes lysine residues
- Rigid polymeric architecture provides high surface area and pore volume, resulting in higher binding capacity, higher flow rates and overall superior performance in affinity chromatography applications
- Beads are incompressible, allowing compatibility with high-pressure chromatography
- Can couple ligands with very high yields in one hour
- Beads have a 60 µm diameter and provide flow-through rates of 3,000 cm/hour
- Accurate gel volumes – just weigh out the support and add the ligand solution directly to the beads; no pre-swelling is necessary
- Compatible with many buffers and pH values
- Superior storage stability with dry powder being stable for at least 18 months
- Excellent reusability – more than 100 cycles with 99% capacity
- Immobilize 1-30 mg protein or 0.1-2 mg peptide/ml resin



UltraLink® *bis*-acrylamide Support with Azlactone Groups | Amine Containing Ligand | Amide Bond Formation with Ring Opening

Ordering Information

Product #	Description	Pkg. Size
46500	**UltraLink® Immobilization Kit**	Kit
	Includes: UltraLink® Biosupport Medium (Will yield approximately 5 x 2 ml columns)	5 x 0.25 g
	Plastic Columns	5 each
	Serum Separator	1 each
	Phosphate Buffered Saline	1 pkg.
	Citrate-Carbonate Buffer	1 pkg.
	Citrate-MOPS Buffer	1 pkg.
	Quenching Buffer	25 ml
	Wash Solution	120 ml
	BCA Protein Assay Reagents:	
	BCA Reagent A	100 ml
	BCA Reagent B	6 ml
46501	**UltraLink® Immobilization Trial Kit**	Trial Kit
	Includes: UltraLink® Biosupport Medium Reagents and Buffers	1 x 0.25 g
53112	**UltraLink® Biosupport Medium (2 ml)**	0.25 g
53110	**UltraLink® Biosupport Medium (8-10 ml)** *Includes disposable column trial kit.*	1.25 g
53111	**UltraLink® Biosupport Medium (50 ml)** *Includes disposable column trial kit.*	6.25 g
28388	**BupH™ Citrate-Carbonate Buffer Packs**	10 packs
28386	**BupH™ Citrate-MOPS Buffer Packs**	10 packs

References

1. Ju, T., *et al.* (2002). *J. Biol. Chem.* **277**, 169-177.
Ju, T., *et al.* (2002). *J. Biol. Chem.* **277**, 178-186.
Kornfeld, R., *et al.* (1998). *J. Biol. Chem.* **273**, 23202-23210.
Liu, L.A. and Engvall, E. (1999). *J. Biol. Chem.* **274**, 38171-38176.

Reacti-Gel® CDI Supports

Offer mild coupling conditions and excellent stability.

Highlights:

- Immobilization occurs through the reaction of an *N*-nucleophile with the imidazolyl carbamate of the Reacti-Gel® Support to form a stable, uncharged *N*-alkylcarbamate linkage – the result is a support free of nonspecific binding
- Imidazolyl-carbamate group couples most efficiently to free amino containing ligands at a pH range of 9-11; optimal coupling yields usually occur at a pH of between 9.5 and 10; many pH-sensitive proteins can be coupled at pH 8.5 in 0.1 M borate buffer
- Reacti-Gel® Supports stored refrigerated at 5°C as an acetone slurry retain full activity for several months
- Reacti-Gel® Supports are available with two different matrices CDI-activated 6% cross-linked beaded agarose
- Highly activated imidazolyl-carbamate matrix
- Exceptionally easy-to-use activated agarose has a half-life measured in hours – hydroxysuccinimide ester-activated supports have half-lives measured in minutes
- Stability of the imidazolyl carbamate group simplifies the filtration and washing before adding a ligand or protein
- Immobilize 1-10 mg protein or 0.1-2 mg peptide/ml resin

CDI-Activated Trisacryl® GF-2000

- Free of interfering charge effects
- Low nonspecific binding
- Rigid matrix allows for high flow rates
- Hydrophilic matrix

References

Shenoy, S.K., *et al.* (2001). *Science* **294**, 1307-1313.
Richardson, R.T., *et al.* (2000). *J. Biol. Chem.* **275**, 30378-30386.

Ordering Information

Product #	Description	Pkg. Size
20259	**Reacti-Gel® (6X) Support** 1,1′-Carbonyldiimidazole activated 6% cross-linked beaded agarose Supplied: stabilized in acetone slurry Agarose hydrated particle size: 45-165 µm Activation level: > 50 µmoles/ml of gel	10 ml
20260	**Reacti-Gel® (6X) Support**	50 ml
20377	**Reacti-Gel® (GF-2000) Support**	50 ml

SulfoLink® Kit and SulfoLink® Coupling Gel

A unique activated support that reacts specifically with reduced sulfhydryl groups.

Highlights:

- Specificity – a variety of proteins can be immobilized via cysteine residues
- SulfoLink® Chemistry is specific for free thiols only; the iodoacetyl functional group assures an irreversible linkage
- Support is 6% cross-linked beaded agarose with a coupling capacity of 5 mg/ml reduced human IgG per ml of gel, or 2 mg of sulfhydryl-containing peptide per ml of gel
- Activated gel contains a 12-atom spacer arm
- Ideal for immobilizing peptides that contain free sulfhydryls or are synthesized with a terminal cysteine residue; use Ellman's Reagent to quantitate the number of available sulfhydryl groups
- Speed – couple and separate in the same column
- Free sulfhydryl groups can be generated by reducing disulfide bonds with mercaptoethanol, DTT, TCEP or mercaptoethyl-amine (MEA)
- In the case of IgG, MEA can reduce the disulfide bond in the hinge region without dissociating heavy and light chains – this leaves the binding site available to interact with antigen in the mobile phase of the chromatography

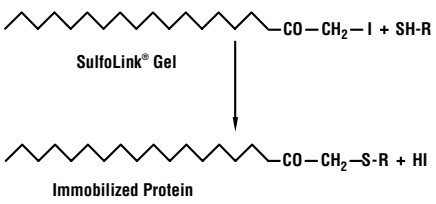
Table 7. Coupling of -SH-/S-S-Containing Proteins to SulfoLink® Coupling Gel Columns

Protein	M.W.	-SH Groups/ Molecules	S-S Groups/ Molecules	Coupling Efficiency
Ceruloplasm	150,000	1 and 3	—	75%
Aldolase	147,000	7 and 28	—	87%
BSA	66,000	0.7	17	25%
HSA	66,000	0.7	17	72%
Ovalbumin	45,000	3 and 4	1	40%
β-Lactoglobin	36,000	2	2	63%
Trypsin	24,000	0	6	13%
Thioredoxin	11,700	2	1	20%
[Arg^8]Vasopressin	1,084	0	1	90%

A variety of proteins can be immobilized to the SulfoLink® Matrix through -SH groups. Eight mg of all proteins were immobilized to 2 ml SulfoLink® Coupling Gel columns according to the coupling protocol. Two mg of the reduced form of the peptide, [Arg^8]Vasopressin, in 2 ml of SulfoLink® Coupling Buffer, was applied to a SulfoLink® Column.

Note: Both BSA and Trypsin were turbid in the Incubation Buffer.

SulfoLink® Coupling Chemistry



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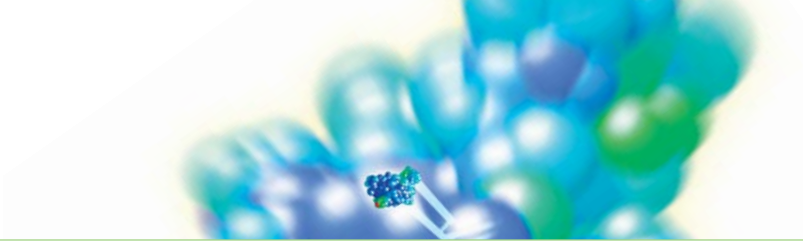
Ordering Information

Product #	Description	Pkg. Size
44895	**SulfoLink® Kit**	Kit
	Includes: SulfoLink® Coupling Gel Columns	5 x 2 ml
	SulfoLink® Preparation Buffer	7.5 ml
	SulfoLink® Coupling Buffer	500 ml
	SulfoLink® Wash Solution	120 ml
	SulfoLink® Reductant	5 x 6 mg
	SulfoLink® Blocking Reagent	100 mg
	Reusable Desalting Column	5 x 5 ml
20405	**SulfoLink® Trial Kit**	Trial Kit
	Includes: Pre-packed Column of SulfoLink® Gel	1 x 2 ml
	Buffers and Reagents	
20401	**SulfoLink® Coupling Gel**	10 ml
20402	**SulfoLink® Coupling Gel**	50 ml



UltraLink® Iodoacetyl Gel

Sulfhydryl reactivity on a durable support.

Highlights:

- UltraLink® Biosupport Medium is activated to yield a terminal iodoacetyl group
- Reacts preferentially with sulfhydryl groups using appropriate coupling conditions
- 15-atom spacer arm reduces steric hindrance that could be associated with a support without a spacer arm and makes binding more efficient
- Low nonspecific binding[1]
- Can couple approximately 8 mg of reduced human IgG per ml of gel, or 2 mg of sulfhydryl-containing peptide per ml of gel

Reference

1. Ju, T., *et al.* (2002). *J. Biol. Chem.* **277**, 169-177.
Hill, K., *et al.* (2000). *J. Biol. Chem.* **275(6)**, 3741-3744.
Liu, L.A. and Engvall, E. (1999). *J. Biol. Chem.* **274**, 38171-38176.
Bicknell, A.B., *et al.* (2001). *Cell* **105**, 903-912.

Ordering Information

Product #	Description	Pkg. Size
53155	**UltraLink® Iodoacetyl Gel** *Includes disposable column trial kit.* Support: UltraLink® Biosupport Medium	10 ml

Disulfide Reducing Agents

Reduce disulfide bonds to produce sulfhydryl groups for immobilization on SulfoLink® Coupling Gel or UltraLink® Iodoacetyl Gel.

Free sulfhydryls are required for immobilization onto sulfhydryl-reactive affinity supports. Cysteines in proteins and peptides usually exist as cystines (disulfide bridges) and must be reduced to expose sulfhydryls for coupling. Reduction may be accomplished with free or immobilized reducing agents. Free reducing agents are efficient in reducing all disulfides in proteins, including those buried in the tertiary structure, but they must be removed from the reduced sample with a desalting column before coupling to the support. Immobilized reducing agents enable reduction of disulfides and simple removal of the reduced sample from the reducing agent. This is especially helpful when reducing peptides whose small size prevents them from being effectively desalted.

Ordering Information

Product #	Description	Pkg. Size
20408	**2-Mercaptoethylamine•HCl**	6 x 6 mg
20290	**DTT, Cleland's Reagent** (Dithiothreitol)	5 g
20291	**No-Weigh™ Dithiothreitol (DTT)**	7.7 mg DTT/ tube x 48 tubes
77700	**Reduce-Imm™ Reducing Kit** *Sufficient reagents to reduce 150 µmoles of disulfide.* Includes: Reduce-Imm™ Immobilized Reductant Columns (capacity: 30-40 µmoles of sulfhydryl groups per column) Equilibration Buffer #1 Equilibration Buffer #2 DTT (used to regenerate and prime the column) Ellman's Reagent (DTNB)	Kit 2 x 2 ml 500 ml 250 ml 250 mg 500 mg
23460	**Protein-Coupling Handle Addition Kit** *Adds free sulfhydryl groups to proteins.* Includes: SATA Hydroxylamine•HCl 10X Conjugation Buffer Stock BupH™ Pack PBS Dimethylformamide (DMF) D-Salt™ Dextran Desalting Column Column Extender Ellman's Reagent (DTNB) Cysteine•HCl H_2O	Kit 2 mg 5 mg 20 ml 1 pack 1 ml 1 x 5 ml 1 2 mg 20 mg
22582	**Ellman's Reagent** (5,5′-Dithio-*bis*-[2-nitrobenzoic acid])	5 g
20490	**TCEP•HCl** (Tris[2-carboxyethyl]phosphine hydrochloride)	1 g
77720	**Bond-Breaker® TCEP Solution, Neutral pH**	5 ml
77712	**Immobilized TCEP Disulfide Reducing Gel**	5 ml

CarboLink™ Kit, CarboLink™ Coupling Gel and UltraLink® Hydrazide

Allow orientation of antibodies through carbohydrates in the hinge region.

Highlights:

- CarboLink™ Gel is 6% beaded agarose derivatized to yield a terminal hydrazide group; UltraLink® Hydrazide is prepared using UltraLink® Biosupport Medium
- Coupling capacity of CarboLink™ Gel is 5-10 mg IgG per 2 ml column; contains a 23-atom spacer arm
- Ideal for polyclonal antibody immobilization[1,2]
- No excess reducing agents are necessary to stabilize the linkage because the resonance structure of the hydrazone bonds are sufficiently stable, resulting in a reusable immunoaffinity column for antigen purification
- Glycoproteins may also be coupled to the CarboLink™ Gel or UltraLink® Hydrazide
- First step is oxidation of the sugar groups, which allows the *cis*-diols of the IgG to be transformed into reactive aldehyde moieties; these aldehydes then combine with hydrazide groups on the matrix to form stable, leak-resistant linkages
- CarboLink™ Gel and UltraLink® Hydrazide react with IgG in the Fc region, so both antigen-binding sites are free to interact with the antigen in the mobile phase

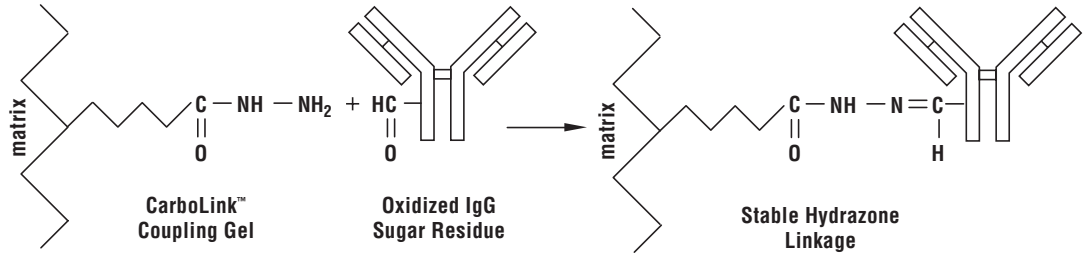
References

1. Kumar, P.G., *et al.* (2001). *J. Biol. Chem.* **276**, 41357-41364.
2. Strakova, Z., *et al.* (1997). *Mol. Pharmacol.* **51**, 217-224.
Brown, M.A., *et al.* (2000). *J. Biol. Chem.* **275**, 19795-19802.
Butko, P., *et al.* (1999). *J. Immunol.* **163**, 2761-2768.
Segura, M., *et al.* (1999). *Infect. Immun.* **67(9)**, 4646-4654.

Ordering Information

Product #	Description	Pkg. Size
44900	**CarboLink™ Kit**	Kit
	Includes: CarboLink™ Coupling Gel Columns	5 x 2 ml
	CarboLink™ Coupling Buffer	250 ml
	CarboLink™ Wash Buffer	100 ml
	CarboLink™ Oxidant	5 x 5 mg
	Disposable Desalting Columns	5 x 5 ml
20390	**CarboLink™ Trial Kit** *Sufficient reagents and buffers for preparing 1 x 2 ml immunoaffinity column.*	Trial Kit
20391	**CarboLink™ Coupling Gel**	10 ml
53149	**UltraLink® Hydrazide** *Includes disposable column trial kit.* Support: UltraLink® Biosupport Medium Spacer Arm: 22 atom Capacity: ≥ 15 µmoles functionality/ml of gel	10 ml
20504	**Sodium *meta*-Periodate**	25 g

Attachment of Oxidized IgG to CarboLink™ Coupling Gel



CarboxyLink™ Coupling Kit and UltraLink® EDC/DADPA Immobilization Kit

Perform peptide immobilization easily.

Highlights:

- Immobilized Diaminodipropylamine contains a nine-atom spacer arm and UltraLink® DADPA contains a 12-atom spacer arm for reduced steric hindrance
- Most peptides and proteins contain exposed carboxyl groups, so carbodiimides like EDC are useful reagents for linking carboxyl groups to primary amine-containing matrices
- Ligands containing a free carboxyl group can be attached by water-soluble carbodiimide coupling procedures, forming an amide linkage
- Coupling can be performed in 50% organic solvent if your peptide is not soluble in aqueous buffer
- EDC has been used in peptide synthesis, immobilization techniques, hapten carrier conjugation, subunit studies and protein:protein interactions
- Spacer arm can be readily extended by reaction with succinic anhydride
- Reaction times for coupling are generally one to three hours
- Immobilize 1-10 mg protein or 0.1-2 mg peptide/ml resin

Reference
Yoo, B.C., *et al.* (2002). *J. Biol. Chem.* **277**, 15325-15332.

Ordering Information

Product #	Description	Pkg. Size
44899	**CarboxyLink™ Immobilization Kit**	Kit
	Includes: Diaminodipropylamine Columns	5 x 2 ml
	EDC	5 x 60 mg
	Coupling Buffer	500 ml
	Wash Buffer	120 ml
	Accessories	
20266	**CarboxyLink™ Coupling Gel**	25 ml
	Support: Cross-linked 4% beaded agarose	
	Loading: 16-20 µmoles available amino groups/ml of gel	
53154	**UltraLink® EDC/DADPA Immobilization Kit**	Kit
	Includes: UltraLink® Immobilized DADPA Columns	5 x 2 ml
	EDC	5 x 60 mg
	Coupling Buffer	500 ml
	Wash Buffer	120 ml
	Porous Polyethylene Discs	6
	Serum Separator	
22980	**Ethylenediamine Dihydrochloride**	5 g
28390	**BupH™ MES Buffered Saline Packs**	10 pack

PharmaLink™ Immobilization Kit

Immobilizes ligands that contain no easily reactive functional groups.

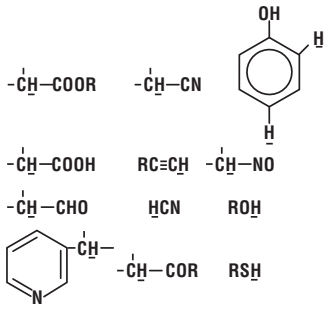
Highlights:

- High-efficiency coupling[1]
- Utilizes the Mannich reaction to immobilize a ligand to a chromatographic support; certain active hydrogens can be condensed with formaldehyde and an amine-containing support
- Hydrogens in ketones, esters, phenols, acetylenes, α-picolines, quinaldines and other compounds may be aminoalkylated using the Mannich reaction
- Useful for immobilizing certain drugs, steroidal compounds, dyes and other organic molecules that contain no available "handles" for easy immobilization, or that have functional groups with low reactivity or that are sterically hindered
- Mannich reaction provides a welcome alternative to the seldom-used diazonium coupling method; Mannich condensations result in stable covalent bonds suitable for the most critical affinity separations
- Immobilize up to 20 µmol/ml resin

An Example of the Mannich Reaction

Reference
1. Rao, M.N., *et al.* (1997). *J. Biol. Chem.* **272**, 24455-24460.

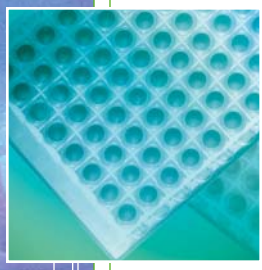
Examples of Active Hydrogens that can Participate in the Mannich Reaction



Ordering Information

Product #	Description	Pkg. Size
44930	**PharmaLink™ Immobilization Kit***	Kit
	Includes: PharmaLink™ Columns	5 x 2 ml
	PharmaLink™ Coupling Buffer	50 ml
	PharmaLink™ Coupling Reagent	4 ml
	PharmaLink™ Wash Buffer	240 ml
	Accessories	
77167	**PharmaLink™ Coupling Buffer**	30 ml
77168	**PharmaLink™ Coupling Reagent**	4 ml

**U.S. Patent # 5,142,027*



Reacti-Bind™ Maleic Anhydride Plates

Proteins and other primary amine-containing compounds covalently attach to the microplate.

Great for immobilization of compounds that do not normally stick to plain polystyrene plates.

Highlights:

- Spontaneously reacts with primary amines
- Maleic anhydride retains its integrity and coupling availability for months

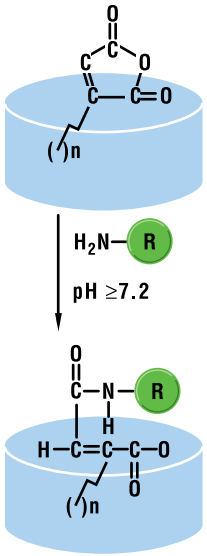
Reference

Brett, P.J., *et al.* (2002). *J. Biol. Chem.* **277**, 20468-20476.

Ordering Information

Product #	Description	Pkg. Size
15110	Reacti-Bind™ Maleic Anhydride Activated Polystyrene 96-Well Plates	5 plates
15112	Reacti-Bind™ Maleic Anhydride Activated Polystyrene 96-Well Plates	25 plates
15100	Reacti-Bind™ Maleic Anhydride Activated Polystyrene Strip Plates	5 plates
15102	Reacti-Bind™ Maleic Anhydride Activated Polystyrene Strip Plates	25 plates

Reaction Scheme for Coupling both Large and Small Amine-Containing Molecules



❶ Maleic Anhydride shown anchored to polystyrene well of a microplate. Both the well wall and base are activated.

❷ R

- Antigen
- Antibody
- Enzyme
- Peptide
- Protein
- Ligand

❸ Stable amide bond formation with amines, with a resulting hydrophilic well surface.

Reacti-Bind™ Maleimide Activated Plates

A convenient alternative to amine-reactive chemistries for attaching sulfhydryl-containing compounds.

Maleimide groups specifically and covalently conjugate sulfhydryl groups at neutral pH, creating a stable thioether bond.

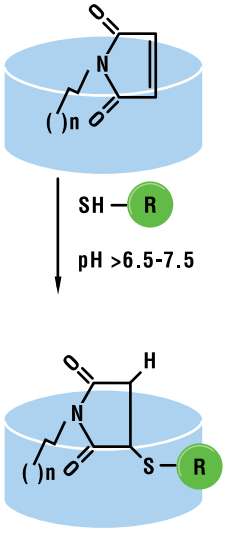
Highlights:

- Pre-blocked with BSA to reduce nonspecific binding
- Convenient 8-well strip format
- Easy (spontaneous) immobilization of peptides derivatized with a terminal cysteine and proteins with free sulfhydryl

Ordering Information

Product #	Description	Pkg. Size
15150	Reacti-Bind™ Maleimide Activated Plates (8-Well Strip)	5 plates

Reaction Scheme for Coupling both Large and Small Sulfhydryl-Containing Molecules



❶ Maleimide shown anchored to polystyrene well of a microplate. Both the well wall and base are activated.

❷ R

- Antigen
- Antibody
- Enzyme
- Peptide
- Protein
- Ligand

❸ Stable thioether bond formation with sulfhydryls, with a resulting hydrophilic well surface.

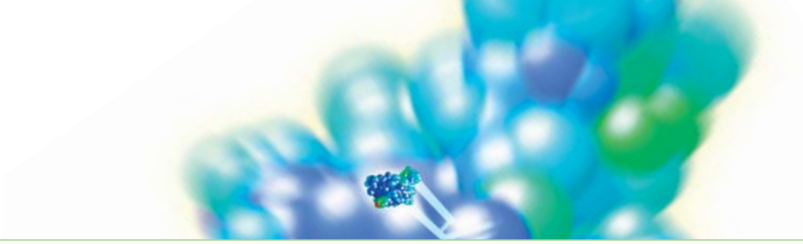
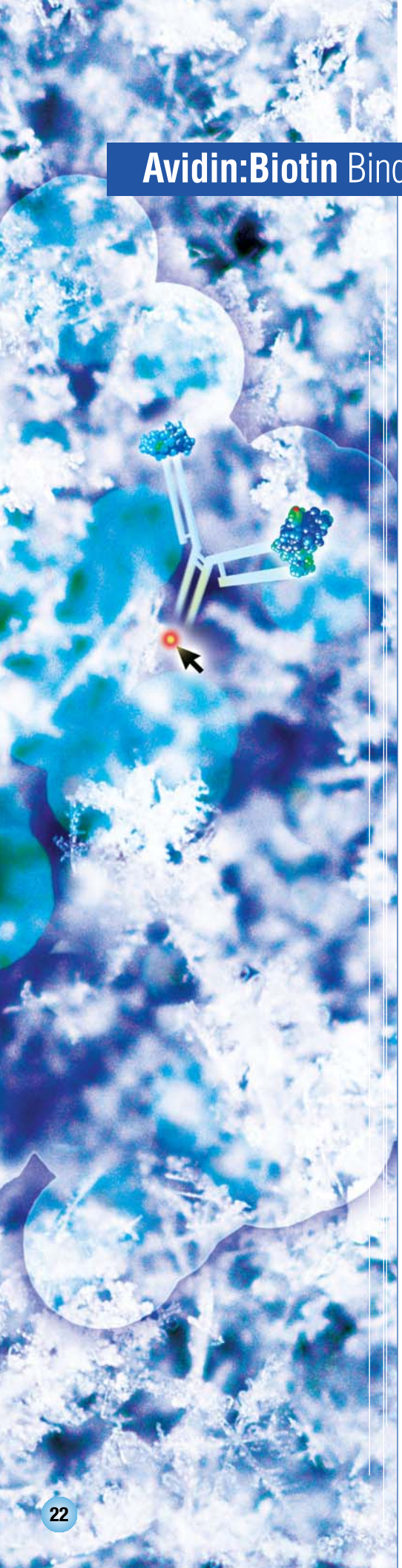


Table 7. Antibody Immobilization: Choosing the Best Support

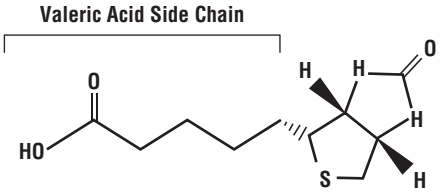
	AminoLink® Plus Coupling Gel	UltraLink® Biosupport Medium	CarboLink™ Coupling Gel or UltraLink® Hydrazide	SulfoLink® Coupling Gel or UltraLink® Iodoacetyl	Orientation Kits (Protein A or Protein G)
Monoclonal Antibodies	**Advantages:** • Good choice when only small amounts of antibody are available • Couple over a broad pH range • Good coupling efficiency	**Advantages:** • Good choice if antibody can withstand 1.0 M sodium citrate or sulfate • High capacity • Fast, efficient coupling • Good, for large-scale or fast-flow applications	**Advantages:** • Correctly orients antibody • Antibody must be able to withstand oxidation conditions • Good for antibodies with low avidity for antigen	**Advantages:** • Good choice for antibodies that have extremely high avidity for their antigen • Allows for gentle elution conditions	**Advantages:** • Allows for correct orientation of antibodies • Gentle coupling conditions • Either Protein A or G will bind most antibodies
	Disadvantages: • Reduction of Schiff's base with sodium cyanoboro-hydride may adversely affect monoclonals • Some antibodies may be coupled through antigen-binding site	**Disadvantages:** • Some antibodies may be coupled through antigen-binding site • Some antibodies may precipitate in high-salt buffer	**Disadvantages:** • Not all monoclonals have carbohydrate accessible for coupling • Conditions necessary for coupling may adversely affect some monoclonals	**Disadvantages:** • Must first reduce antibody prior to coupling • Not good for antibodies with low affinity for their antigens	**Disadvantages:** • If purifying antigen from serum, antibodies may bind to remaining Protein A or G site and co-purify with antigen
Polyclonal Antibodies	**Advantages:** • Excellent coupling efficiency • Good antigen recovery	**Advantages:** • Good choice for large-scale or fast-flow applications • High capacity • Fast, efficient coupling	**Advantages:** • Correctly orients antibody • Antibody must be able to withstand oxidation conditions • Good for antibodies with low avidity for antigen	**Advantages:** • Good choice for antibodies that have avidity for their antigen • Allows for gentle elution conditions	**Advantages:** • Allows for correct orientation of antibodies • Gentle couple conditions • Either Protein A or G will bind most antibodies
	Disadvantages: • Some antibodies may be coupled through antigen-binding site	**Disadvantages:** • Some antibodies may be coupled through antigen-binding site • Some antibodies may precipitate in high-salt buffer	**Disadvantages:** • Conditions necessary for coupling may adversely affect some antibodies	**Disadvantages:** • Must first reduce antibody prior to coupling • Not good for antibodies with low affinity for their antigens	**Disadvantages:** • If purifying antigen from serum, antibodies may bind to remaining Protein A or G sites and co-purify with antigen
High-Avidity Antibodies				**Advantages:** • Immobilization of reduced antibody allows for gentler elution conditions	
Low-Avidity Antibodies			**Advantages:** • Correctly orients antibody		**Advantages:** • Allows for correct orientation of antibodies • Gentle couple conditions • Either Protein A or Protein G will bind most antibodies
			Disadvantages: • Conditions necessary for coupling may adversely affect some monoclonals		**Disadvantages:** • If purifying antigen from serum, antibodies may bind to remaining Protein A and Protein G sites and co-purify with antigen



Avidin:Biotin Binding

Biotin

Biotin, also known as vitamin H, is a small molecule (MW 244.3) that is present in tiny amounts in all living cells. The valeric acid side chain of the biotin molecule can be derivatized to incorporate various reactive groups that are used to attach biotin to other molecules. Once biotin is attached to a molecule, the molecule can be affinity-purified using an immobilized version of any biotin-binding protein. Alternatively, a biotinylated molecule can be immobilized through interaction with a biotin-binding protein, then used to affinity-purify other molecules that specifically interact with it. Pierce offers biotin-labeled antibodies and a number of other biotinylated molecules, as well as a broad selection of biotinylation reagents to label any protein.

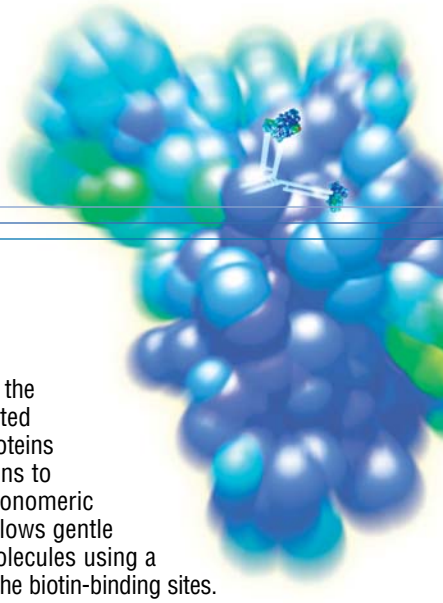


Biotin
M.W. 244.3

Biotin-Binding Proteins

Avidin – The extraordinary affinity of avidin for biotin allows biotin-containing molecules in a complex mixture to be discretely bound with avidin. Avidin is a glycoprotein found in the egg white and tissues of birds, reptiles and amphibia. It contains four identical subunits having a combined mass of 67,000-68,000 daltons. Each subunit consists of 128 amino acids and binds one molecule of biotin. The extent of glycosylation on avidin is high; carbohydrate accounts for about 10% of the total mass of the tetramer. Avidin has a basic isoelectric point (pI) of 10-10.5 and is stable over a wide range of pH and temperature. Extensive chemical modification has little effect on the activity of avidin, making it especially useful for protein purification. However, because of its carbohydrate content and basic pI, avidin has relatively high nonspecific binding properties.

Streptavidin – Another biotin-binding protein is streptavidin, which is isolated from *Streptomyces avidinii* and has a mass of 75,000 daltons. In contrast to avidin, streptavidin has no carbohydrate and has a mildly acidic pI of 5.5. Pierce products use a recombinant form of streptavidin having a mass of 53,000 daltons and a near-neutral pI. Streptavidin is much less soluble in water than avidin. There are considerable differences in the composition of avidin and streptavidin, but they are remarkably similar in other respects. Streptavidin is also a tetrameric protein, with each subunit binding one molecule of biotin with affinity similar to that of avidin. Guanidinium chloride will dissociate avidin and streptavidin into subunits, but streptavidin is more resistant to dissociation. Streptavidin contains a RYD sequence similar to the RGD sequence that binds cell surface receptors. The RYD sequence can cause background in some applications.



NeutrAvidin™ Protein – Pierce also offers a deglycosylated version of avidin, known as NeutrAvidin™ Biotin-Binding Protein, with a mass of approximately 60,000 daltons. As a result of carbohydrate removal, lectin binding is reduced to undetectable levels, yet biotin-binding affinity is retained because the carbohydrate is not necessary for this activity. NeutrAvidin™ Protein offers the advantages of a near-neutral pI (6.3) to minimize nonspecific adsorption, along with lysine residues that remain available for derivatization or conjugation. NeutrAvidin™ Protein yields the lowest nonspecific binding among the known biotin-binding proteins due to its near-neutral pI and lack of both carbohydrate and RYD sequence.

Strength of Avidin-Biotin Interaction – The avidin-biotin complex is the strongest known noncovalent interaction ($K_a = 10^{15}\ M^{-1}$) between a protein and ligand. The bond formation between biotin and avidin is rapid and, once formed, is unaffected by extremes of pH, temperature, organic solvents and most denaturing agents. These features of avidin – features that are shared by streptavidin and NeutrAvidin™ Protein – make immobilized forms of the biotin-binding proteins particularly useful for purifying biotin-labeled proteins or other molecules. However, the strength of the interaction and its resistance to dissociation make it difficult to elute bound proteins from an immobilized support. Harsh, denaturing conditions (8 M guanidine•HCl, pH 1.5 or boiling in SDS-sample loading buffer) are required to efficiently dissociate avidin:biotin complexes. Such conditions damage the support irreversibly so that it cannot be reused, and denature the eluted proteins so that they do not maintain any biological activity.

Because of these binding and elution properties, purifications based on avidin:biotin affinity are reserved primarily for small-scale procedures involving immediate analysis of the eluted sample by reducing SDS-PAGE or other denaturing method. On the other hand, it is possible to take advantage of the strong avidin:biotin binding properties in immunoprecipitation (IP) and pull-down procedures because the immunoprecipitated "prey" protein can be recovered using elution conditions that will not also elute the biotinylated antibody or "bait" protein. In some situations, it may be most appropriate to use a cleavable biotinylation reagent to label the target molecule so that it may be recovered from its bound state to immobilized avidin by specific cleavage of the spacer arm between biotin and target molecule rather than by elution of biotin from avidin.

Monomeric Avidin – Pierce developed Immobilized Monomeric Avidin to allow the purification of fully functional biotinylated proteins. Unlike other biotin-binding proteins that require harsh, denaturing conditions to elute and recover bound molecules, Monomeric Avidin binds reversibly to biotin and allows gentle elution and recovery of biotinylated molecules using a solution of 2 mM biotin to compete for the biotin-binding sites. This makes it possible to harness the avidin:biotin interaction as a purification tool to recover functional proteins and other biological molecules.

Biotin-Binding Products

Each of the four biotin-binding proteins discussed is available in a variety of immobilized formats. The support used for ImmunoPure® Immobilized Avidin, Streptavidin and NeutrAvidin™ Protein is a 6% cross-linked, beaded agarose resin. ImmunoPure® Immobilized Monomeric Avidin uses a 4% cross-linked, beaded agarose resin. The UltraLink® Resin is a durable, polyacrylamide-based support with a high surface area, large pore volume and low nonspecific binding. It is suitable for pressures up to 100 psi and linear flow rates up to 3,000 cm/hour. A biotin-binding protein immobilized on agarose or UltraLink® Beads may be used for affinity purification in a column or batch method. NeutrAvidin™ Protein and Streptavidin are also available bound to polystyrene microplates along with a dried blocking buffer. These 96-well plates are offered in transparent, white or black plates to accommodate a variety of assay types. The plates come in two forms – regular and high-binding capacity. The high-binding capacity plates contain more of the immobilized NeutrAvidin™ Protein or Streptavidin and are ideal for binding large amounts of small, biotin-containing molecule (e.g., a biotinylated peptide). Streptavidin immobilized on MagnaBind™ Magnetic beads is an excellent tool for cell-sorting applications.

Avidin:Biotin Binding

A Comparison of the Biotin-Binding Proteins

The strong association between avidin and biotin can be used in the field of affinity separations. By attaching avidin to a solid support, a biotinylated product can be anchored to the same solid support. The attachment is stable over a wide range of pH, salt concentrations and temperatures. To dissociate biotin from avidin, 8 M guanidine•HCl, pH 1.5 or boiling in SDS-PAGE sample buffer must be used.

Protein	Isoelectric Point	Contains Carbohydrate	Nonspecific Binding	MW	RYD Sequence
Avidin	10-10.5	Yes	High	67 kDa	No
Streptavidin	5.5	No	Low	75 kDa	Yes
NeutrAvidin™ Biotin-Binding Protein	6.3	No	Ultralow	60 kDa	No

Immobilized Avidin Products

Strong biotin interaction creates a nearly irreversible bond.

Immobilized avidin can be used in a variety of applications for the affinity purification of biotinylated macromolecules. In one variation, an antibody that has an affinity for a particular antigen is labeled with biotin. Cells containing the antigen are lysed, then incubated with the biotinylated antibody to form a typical antigen/antibody complex. To isolate the antigen, the crude mixture is passed through an immobilized avidin or streptavidin column, which will bind the complex. After appropriate washes, the antigen can be eluted from the column with a low pH elution buffer. The biotinylated antibody is retained by the column.

Applications:

- Binding biotinylated anti-transferrin for purifying transferrin from serum[1]
- Binding biotinylated peptides and elution with an SDS/urea solution[2]
- Hybridization of biotinylated RNA to its complementary DNA and binding to immobilized avidin, with subsequent elution of the single-stranded DNA[3]
- Purification of double-stranded DNA[4]

References

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3. Manning, J., *et al.* (1977). *Biochemistry* **16**, 1364-1370.
4. Pellegrini, M., *et al.* (1977). *Nucleic Acids Res.* **4**, 2961-2973.

Claypool, S.M., *et al.* (2002). *J. Biol. Chem.* **277**, 28038-28050.
Sharma, K.K., *et al.* (2000). *J. Biol. Chem.* **275**, 3767-3771.
Wilchek, M. and Bayer, E.A. (1989). Protein Recognition of Immobilized Ligands. Hutchins, T.W., ed. Alan R. Liss, Inc., pp. 83-90.

Ordering Information

Product #	Description	Pkg. Size
20219	**ImmunoPure® Immobilized Avidin Gel** Support: Cross-linked 6% beaded agarose Spacer: none (directly attached) Supplied: 50% aqueous slurry containing 0.02% sodium azide	5 ml
20362	**AffinityPak™ Immobilized Avidin Columns**	5 x 1 ml
20225	**ImmunoPure® Immobilized Avidin Gel**	5 x 5 ml

Immobilized Streptavidin Products

Same high biotin-binding affinity as avidin with low nonspecific binding.

Applications:

- Purification of membrane antigens in conjunction with biotinylated monoclonal antibodies[1,2]
- Cell-surface labeling with biotinylation reagents, followed by precipitation with immobilized streptavidin[3]
- Purification of cell-surface glycoproteins using biotinylated Concanavalin A[4]
- Recovery of single-stranded DNA for dideoxy sequencing[5]

References

1. Gretch, D.R., *et al.* (1987). *Anal. Biochem.* **163**, 270-277.
2. Updyke, T.V. and Nicolson, G.L. (1984). *J. Immunol. Method* **73**, 83-95.
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4. Buckie, J.W. and Cook, G.M. (1986). *Anal. Biochem.* **156(2)**, 463-472.
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Ordering Information

Product #	Description	Pkg. Size
20347	**ImmunoPure® Immobilized Streptavidin Gel** Support: Cross-linked 6% beaded agarose Capacity: Approximately 1-3 mg biotinylated BSA/ml of gel	2 ml
20349	**ImmunoPure® Immobilized Streptavidin Gel**	5 ml
20351	**AffinityPak™ Immobilized Streptavidin Columns**	5 x 1 ml
53113	**UltraLink® Immobilized Streptavidin Gel** Capacity: ≥2 mg of biotinylated BSA/ml of gel	2 ml
53114	**UltraLink® Immobilized Streptavidin Gel**	5 ml
53116	**UltraLink® Immobilized Streptavidin Plus Gel** Capacity: ≥3 mg of biotinylated BSA/ml of gel	2 ml
53117	**UltraLink® Immobilized Streptavidin Plus Gel**	5 ml
21344	**MagnaBind™ Streptavidin Beads**	5 ml

Immobilized NeutrAvidin™ Products

Less nonspecific binding produces cleaner results and better yields.

Highlights:

- Carbohydrate-free – just like streptavidin, NeutrAvidin™ Biotin-Binding Protein has no carbohydrate, eliminating nonspecific binding problems due to sugars
- No interaction with cell surface molecules – absence of the Arg-Tyr-Asp sequence (present in streptavidin), which mimics the universal cell surface recognition sequence present in a variety of molecules, eliminates cross-reactivity of cell surface molecules
- Neutral pI – with a pI of 6.3, NeutrAvidin™ Biotin-Binding Protein has a pI that is closer to neutrality than avidin or streptavidin, eliminating electrostatic interaction that contributes to nonspecific binding

When nonspecific binding is a problem in your application, Pierce offers a variety of immobilized NeutrAvidin™ products as superior alternatives to avidin or streptavidin. NeutrAvidin™ Biotin-Binding Protein is a modified avidin derivative that combines several key features to provide biotin-binding with exceptionally low nonspecific binding properties.

Applications:

- Immunoprecipitation
- Purifying proteins that bind to biotinylated ligands
- Capturing biotinylated cell-surface proteins[1-3]
- Purifying biotinylated peptides[4]

References

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2. Daniels, G.M. and Amara, S.G. (1998). *Methods Enzymol.* **296**, 307-318.
3. Liaw, P.C.Y., *et al.* (2001). *J. Biol. Chem.* **276**, 8364-8370.
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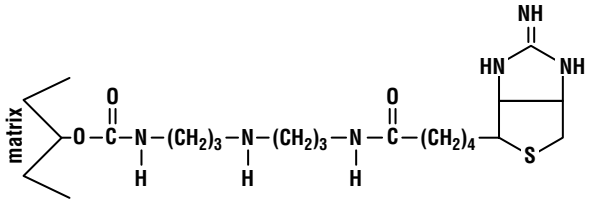
Lesa, G.M., *et al.* (2000). *J. Biol. Chem.* **275**, 2831-2836.
Trotti, D., *et al.* (2001). *J. Biol. Chem.* **276**, 576-582.

Ordering Information

Product #	Description	Pkg. Size
29200	**Immobilized NeutrAvidin™ on Agarose**	5 ml
53150	**UltraLink® Immobilized NeutrAvidin™** *Includes disposable column trial kit.* *Capacity: Approximately 9-12 µg of biotin/ml of gel*	5 ml
53151	**UltraLink® Immobilized NeutrAvidin™ Plus** *Includes disposable column trial kit.* *Capacity: ≥17 µg of biotin/ml of gel*	5 ml

Immobilized Iminobiotin and D-Biotin

Iminobiotin offers mild dissociation conditions at pH 4.



Immobilized Iminobiotin

Iminobiotin is the guanido analog of biotin. The dissociation constant of the avidin-iminobiotin complex is pH-dependent. At pH 9.5-11.0, the avidin-iminobiotin complex will bind tightly. At pH 4, the avidin-iminobiotin complex will dissociate. Because denaturing agents such as 8 M guanidine•HCl or 4 M urea are not used in the purification, an avidin conjugate has a better chance of maintaining its activity during purification.

Use immobilized D-Biotin as an "irreversible linkage" to bind streptavidin conjugates. The biotin-streptavidin interaction can withstand extremes in pH, salt and detergents.

References
Gitlin, G., *et al.* (1987). *Biochem. J.* **242**, 923-926.
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Hofmann, K., *et al.* (1980). *Proc. Natl. Acad. Sci. USA* **77(8)**, 4666-4668.
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Hofmann, K., *et al.* (1980). *Proc. Natl. Acad. Sci. USA* **77**, 4666-4668.

Ordering Information

Product #	Description	Pkg. Size
20221	**Immobilized Iminobiotin Gel** Support: Cross-linked beaded agarose Spacer: Diaminodipropylamine Supplied: 50% aqueous slurry containing 0.02% sodium azide Capacity: ≥1 mg of avidin/ml of gel	5 ml
20218	**Immobilized D-Biotin Gel** Support: Cross-linked 6% beaded agarose Spacer: Diaminodipropylamine Supplied: 50% aqueous slurry containing 0.02% sodium azide Capacity: 2 mg of avidin/ml of gel	5 ml

Immobilized Monomeric Avidin and Kit

Ideal affinity support for gentle, reversible binding of biotinylated proteins.

To break the biotin:avidin interaction, 8 M guanidine•HCl at pH 1.5 or boiling in SDS-PAGE sample buffer is required. These elution methods may result in denaturation of the biotinylated protein and cause irreversible damage to the support. In addition, avidin or streptavidin will be irreversibly denatured and lose the ability to bind subsequent biotinylated samples.

When avidin is coupled to a gel as the subunit monomer, the specificity for biotin is retained, but the affinity for biotin binding substantially decreases (kDa approximately 10^{-8} M). Immobilized Monomeric Avidin and the ImmunoPure® Immobilized Monomeric Avidin Kit can be used to bind biotinylated molecules, and the bound material can be competitively eluted using 2 mM biotin in phosphate buffered saline (PBS). This technique provides the gentlest elution conditions without contamination of the avidin subunits or substantial loss of column-binding capacity.

Highlights:
- Purifies biotinylated products under mild elution conditions
- Can be regenerated and reused at least 10 times
- Exhibits little nonspecific binding (3% or less)

References
Bernstein, E.M., *et al.* (1999). *J. Biol. Chem.* **274(2)**, 889-895.
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Ellerbroek, S.M., *et al.* (2001). *J. Biol. Chem.* **276**, 24833-24842.
Glover, B.P. and McHenry, C.S. (2001). *Cell* **105**, 925-934.
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Oda, Y., *et al.* (2001). *Nature Biotechnology* **19**, 379-382.
Schwarzman, A.L., *et al.* (1999). *Proc. Natl. Acad. Sci. USA* **96**, 7932-7937.
Slatin, S.L., *et al.* (2002). *Proc. Natl. Acad. Sci. USA* **99**, 1286-1291.

Ordering Information

Product #	Description	Pkg. Size
20228	**ImmunoPure® Immobilized Monomeric Avidin Gel** *Includes disposable column trial kit.* Support: Cross-linked 4% beaded agarose	5 ml
20227	**ImmunoPure® Immobilized Monomeric Avidin Kit**	Kit
	Includes: Monomeric Avidin Column	2 ml
	BupH™ Phosphate Buffered Saline Pack (yields 500 ml)	1 pack
	Biotin Blocking and Elution Buffer	200 ml
	Regeneration Buffer	250 ml
	Column Extender	
53146	**UltraLink® Immobilized Monomeric Avidin**	5 ml
29129	**ImmunoPure® D-Biotin**	1 g

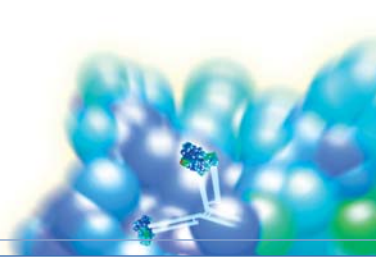


Table 9. Biotinylation Reagent Selection Guide

Product #	Description	Chemical Reactivity	Water-Soluble	Spacer Arm Length	Cleavable	Membrane-Permeable*
21335	Sulfo-NHS-LC-Biotin	Primary Amine	Yes	22.4 Å	No	No
21338	Sulfo-NHS-LC-LC-Biotin	Primary Amine	Yes	30.5 Å	No	No
21217	Sulfo-NHS-Biotin	Primary Amine	Yes	13.5 Å	No	No
21331	Sulfo-NHS-SS-Biotin	Primary Amine	Yes	24.3 Å	Yes	No
21330	EZ-Link® NHS-PEO_4-Biotin	Primary Amine	Yes	32 Å	No	No
21329	No-Weigh™ Pre-Measured NHS-PEO_4-Biotin Microtubes	Primary Amine	Yes	32 Å	No	No
21336	NHS-LC-Biotin	Primary Amine	No	22.4 Å	No	Yes
21343	NHS-LC-LC-Biotin	Primary Amine	No	30.5 Å	No	Yes
20217	NHS-Biotin	Primary Amine	No	13.5 Å	No	Yes
21117	NHS-Iminobiotin TFA	Primary Amine	No	13.5 Å	No	Yes
21218	PFP-Biotin	Primary or Secondary Amines/RNA/DNA	No	9.6 Å	No	Yes
21219	TFP-PEO-Biotin	Primary Amine	Yes	32.6 Å	No	No
21901	Maleimide-PEO_2-Biotin	Sulfhydryl	Yes	29.1 Å	No	No
21902	No-Weigh™ Maleimide-PEO_2-Biotin Microtubes	Sulfhydryl	Yes	29.1 Å	No	No
21900	Biotin-BMCC	Sulfhydryl	No	32.6 Å	No	Yes
21334	PEO-Iodoacetyl Biotin	Sulfhydryl	Yes	24.7 Å	No	No
21333	Iodoacetyl-LC-Biotin	Sulfhydryl	No	27.1 Å	No	Yes
21341	Biotin-HPDP	Sulfhydryl	No	29.2 Å	Yes	Yes
21346	Biotin PEO-Amine	Carboxyl**	Yes	20.4 Å	No	No
21347	Biotin PEO-LC-Amine	Carboxyl**	Yes	22.9 Å	No	No
21345	5-(Biotinamido)pentylamine	Carboxyl**	Yes	18.9 Å	No	No
28020	Biocytin Hydrazide	Carbohydrate/RNA/DNA	Yes	19.7 Å	No	No
21339	Biotin Hydrazide	Carbohydrate	No	15.7 Å	No	Yes
21340	Biotin-LC-Hydrazide	Carbohydrate	No	24.7 Å	No	Yes
29986	Psoralen-PEO-Biotin	DNA/RNA/Protein	Yes	36.9 Å	No	No
22020	PEO-Biotin Dimer	Avidin Cross-linking	Yes	43.4 Å	No	No
29987	Photoactivatable Biotin	DNA/RNA/Protein	No	30.0 Å	No	Yes
29982	Biotin-LC-ASA	DNA/RNA/Protein	No	29.9 Å	No	Yes
28022	Biocytin	Hydrazide	Yes	20.1 Å	No	No
33033	Sulfo-SBED	Trifunctional	Yes	N/A	Yes	No

** Membrane permeability is implied due to a molecule's hydrophobic/hydrophilic nature.*
*** When used with EDC (Product # 22980, 22981).*

Reacti-Bind™ NeutrAvidin™ Coated Polystyrene Plates

The high affinity of avidin for biotin, without the nonspecific binding problems.

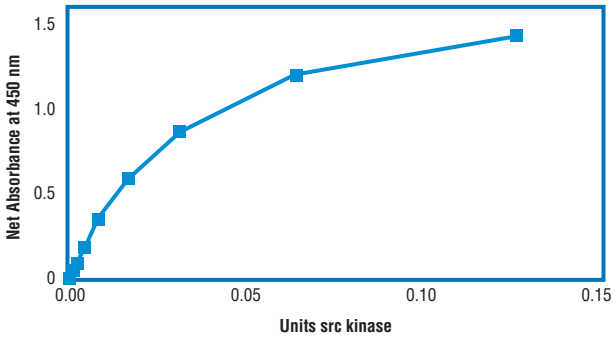
Highlights:

- Easy and gentle immobilization of biotin-containing conjugates
- Lowest nonspecific binding properties of all biotin-binding proteins
- NeutrAvidin™ Biotin-Binding Protein has no carbohydrate and an isoelectric point of 6.3
- No denaturing of the protein component of a conjugate upon binding to the plate
- Ideal for binding small hydrophilic molecules (e.g., peptides) that typically exhibit poor binding directly to polystyrene
- Pre-blocked with your choice of Blocker™ BSA or SuperBlock® Blocking Buffer
- Available in 96- and 384-well formats

Table 10.

Protein	Isoelectric Point	Contains Carbohydrate	Nonspecific Binding
Avidin	10-10.5	Yes	High
Streptavidin	5.5	No	Low
NeutrAvidin™ Biotin-Binding Protein	6.3	No	Ultralow

Purified p60$^{c\text{-}src}$ Activity Detection with TK Peptide 2



Biotinylated tyrosine kinase peptide 2 was added to Reacti-Bind™ NeutrAvidin™ Coated Plates and incubated for 30 minutes. Wells were washed; samples containing p60$^{c\text{-}src}$ tyrosine kinase were added to phosphorylate the tyrosine residue on the peptide. Anti-phosphotyrosine monoclonal antibody conjugated to horseradish peroxidase was added. Tyrosine kinase activity was detected by 1-Step™ Turbo TMB Substrate. Kinase activity was quantitated by comparison with a standard curve generated using the phosphorylated form of the same peptide substrate.

Reference

Singh, Y., *et al.* (1999). *Infect. Immun.* **67**, 1853-1859.

Ordering Information

Product #	Description	Pkg. Size
15129	**Reacti-Bind™ NeutrAvidin™ Coated 96-Well Plates, Clear Plates with SuperBlock® Blocking Buffer**	5 plates
15123	**Reacti-Bind™ NeutrAvidin™ Coated 96-Well Plates, Clear Plates with Blocker™ BSA**	5 plates
15128	**Reacti-Bind™ NeutrAvidin™ Coated Strip Plates, Clear Plates with Blocker™ BSA**	5 plates
15127	**Reacti-Bind™ NeutrAvidin™ Coated Strip Plates, Clear Plates with SuperBlock® Blocking Buffer**	5 plates
15116	**Reacti-Bind™ NeutrAvidin™ Coated 96-Well Plates, White Plates with SuperBlock® Blocking Buffer**	5 plates
15117	**Reacti-Bind™ NeutrAvidin™ Coated 96-Well Plates, Black Plates with SuperBlock® Blocking Buffer**	5 plates
15400	**Reacti-Bind™ NeutrAvidin™ Coated Clear 384-Well Plates with SuperBlock® Blocking Buffer**	5 plates
15401	**Reacti-Bind™ NeutrAvidin™ Coated White 384-Well Plates with SuperBlock® Blocking Buffer**	5 plates
15402	**Reacti-Bind™ NeutrAvidin™ Coated Black 384-Well Plates with SuperBlock® Blocking Buffer**	5 plates
15115	**Reacti-Bind™ Biotin Binding Plate Sample Pack** One each of the following plates: Product #'s 15120, 15121, 15127 and 15128	4 plates

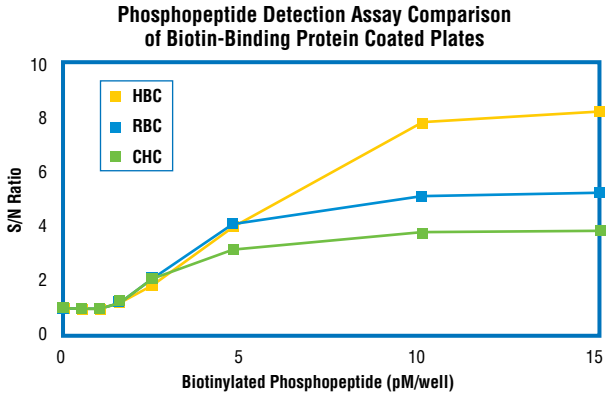
Reacti-Bind™ NeutrAvidin™ High Binding Capacity (HBC) Coated Plates

Unique technology for improved assay precision.

Rely on Pierce to offer researchers a wide variety of Avidin-Biotin products including our exclusive Reacti-Bind™ NeutrAvidin™ Coated Plates available in a high binding capacity (HBC) format. NeutrAvidin™ Biotin-Binding Protein is a deglycosylated form of avidin with a near-neutral pI that results in less nonspecific binding than that of streptavidin or avidin. Pierce's patent-pending plate-coating technology offers a NeutrAvidin™ HBC Plate with a wider detection limit than our regular binding capacity plates. The standard curve exhibits greater linearity for detecting small biotinylated molecules such as peptides (see Figure) and oligonucleotides, resulting in greater assay precision. Try Reacti-Bind™ NeutrAvidin™ HBC Coated Plates for binding small biotinylated ligands and see the difference.

Highlights:

- Unique plate-coating technology – results in high loading of NeutrAvidin™ Biotin-Binding Protein/well
- Improved sensitivity – less nonspecific binding for improved signal-to-noise ratios
- Broader dynamic range – extends the quantitative range so there's no need for dilutions
- Save time – pre-blocked plates to reduce the number of assay steps
- Flexible assay formats – coated plates offered in 96- and 384-well formats and in different colors



Comparison of NeutrAvidin™ High Binding Capacity (HBC) Coated Plate, NeutrAvidin™ Regular Binding Capacity (RBC) Coated Plates and a competitor's Streptavidin Coated High Binding Capacity Plates (CHC). Plates were incubated with various dilutions of biotinylated, phosphorylated peptide. After washing, the plates were incubated with mouse anti-phosphotyrosine antibody (1:1,000) and then detected using an anti-mouse-FITC conjugate (1:666). The Y-axis is described as the signal-to-noise (S/N) ratio.

Table 11. Reacti-Bind™ NeutrAvidin™ HBC Coated Plate Characteristics

	96-Well Plate	384-Well Plate
Binding Capacity	60 pmoles/well	35 pmoles/well
Coat Volume	100 µl/well	50 µl/well
Blocking Volume	200 µl/well	100 µl/well

Ordering Information

Product #	Description	Pkg. Size
15507	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), Clear 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15508	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), Clear 8-Well Strip Plates with SuperBlock® Blocking Buffer	5 plates
15509	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), White 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15510	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), Black 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15511	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), Clear 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15512	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), White 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15513	Reacti-Bind™ NeutrAvidin™ Coated Plate (HBC), Black 384-Well Plates with SuperBlock® Blocking Buffer	5 plates

Reacti-Bind™ Streptavidin Coated Polystyrene Plates

The specific binding affinity of streptavidin for biotin – in a microplate.

Highlights:

- Easy and gentle immobilization of biotin-containing conjugates
- Low nonspecific binding
- No denaturing of the protein component of a conjugate upon binding
- Ideal for binding small biotinylated hydrophilic molecules (e.g., peptides) that typically exhibit poor binding to polystyrene
- Pre-blocked with your choice of Blocker™ BSA or SuperBlock® Blocking Buffer
- Binding capacity for 96-well plates: 5 pmoles of biotin fluorescein per well using a 100 µl coat volume and 200 µl blocking volume
- Binding capacity for 384-well plates: 4 pmoles of biotin fluorescein per well using a 50 µl coat volume and 100 µl blocking volume

Reference

Estrada, G., *et al.* (1996). *Mol. Cell Probes* **10**, 179-185.
Grobler, J.A. *et al.* (2002). *Proc. Nat. Acad. Sci., USA* **99**, 6661-6666.

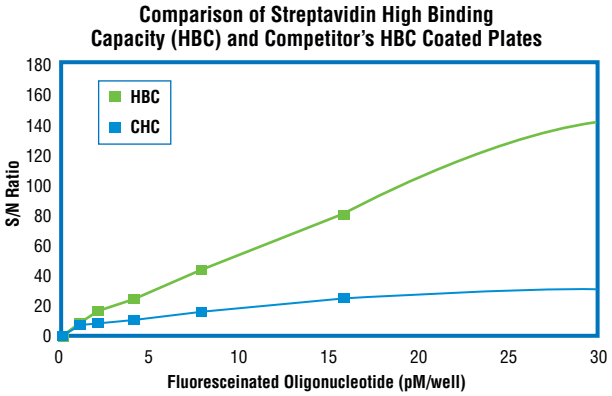
Ordering Information

Product #	Description	Pkg. Size
15120	Reacti-Bind™ Streptavidin Coated Polystyrene Strip Plates with SuperBlock® Blocking Buffer (Clear)	5 plates
15121	Reacti-Bind™ Streptavidin Coated Polystyrene Strip Plates with Blocker™ BSA (Clear)	5 plates
15122	Reacti-Bind™ Streptavidin Coated Polystyrene Strip Plates with SuperBlock® Blocking Buffer (Clear)	25 plates
15124	Reacti-Bind™ Streptavidin Coated 96-Well Plates with SuperBlock® Blocking Buffer (Clear)	5 plates
15125	Reacti-Bind™ Streptavidin Coated 96-Well Plates with Blocker™ BSA (Clear)	5 plates
15126	Reacti-Bind™ Streptavidin Coated Polystyrene 96-Well Plates with SuperBlock® Blocking Buffer (Clear)	25 plates
15118	Reacti-Bind™ Streptavidin Coated White Polystyrene 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15119	Reacti-Bind™ Streptavidin Coated Black Polystyrene 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15115	Reacti-Bind™ Biotin Binding Plate Sample Pack One each of the following plates: Product #'s 15120, 15121, 15127 and 15128	4 plates
15405	Reacti-Bind™ Streptavidin Coated Clear 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15406	Reacti-Bind™ Streptavidin Coated White 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15407	Reacti-Bind™ Streptavidin Coated Black 384-Well Plates with SuperBlock® Blocking Buffer	5 plates

Reacti-Bind™ Streptavidin HBC Coated Plates

Take advantage of a Pierce technology that provides a broader dynamic range.

Reacti-Bind™ Streptavidin High Binding Capacity (HBC) Coated Plates are designed for binding biotinylated oligonucleotides and peptides with higher binding efficiency than other commercially available plates. Pierce's proprietary coating technology (patent pending) has created a streptavidin-coated plate with four- to five-times the binding capacity of competitors' plates. Using a Reacti-Bind™ Streptavidin HBC Plate can result in an assay with a broader dynamic range and better linearity, leading to improved assay precision. The figure below demonstrates this effect when measuring fluorescent polymerase chain reaction (PCR) products hybridized to biotinylated oligonucleotides bound to a Reacti-Bind™ High Binding Capacity (HBC) Plate and a leading competitor's high binding capacity plate (CHC). Try the Reacti-Bind™ Streptavidin HBC Coated Plate and see what has been going undetected in your research.



Comparison of Reacti-Bind™ Streptavidin High Binding Capacity (HBC) Coated Plate with competing high binding capacity plate (CHC). Plates were incubated with a biotinylated oligonucleotide, washed and probed with a complementary oligonucleotide labeled with fluorescein at various dilutions. The Y-axis is described as the signal-to-noise (S/N) ratio.

Highlights:

- Broader dynamic range – extends the quantitative range so there's no need for dilutions
- Better sensitivity – increased binding capacity allows direct detection of small ligands not observed with regular binding capacity plates
- Superior assay precision – standard curve demonstrates greater linearity
- Save time – pre-blocked to reduce number of assay steps
- Flexible assay formats – offered in 96- and 384-well formats and in different colors

Table 12. Reacti-Bind™ Streptavidin HBC Coated Plate Characteristics

	96-Well Plate	384-Well Plate
Binding Capacity	125 pmoles/well	60 pmoles/well
Coat Volume	100 µl/well	50 µl/well
Blocking Volume	200 µl/well	100 µl/well

Ordering Information

Product #	Description	Pkg. Size
15500	Reacti-Bind™ Streptavidin Coated Plates (HBC), Clear 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15501	Reacti-Bind™ Streptavidin Coated Plates (HBC), Clear 8-Well Strip Plates with SuperBlock® Blocking Buffer	5 plates
15502	Reacti-Bind™ Streptavidin Coated Plates (HBC), White 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15503	Reacti-Bind™ Streptavidin Coated Plates (HBC), Black 96-Well Plates with SuperBlock® Blocking Buffer	5 plates
15504	Reacti-Bind™ Streptavidin Coated Plates (HBC), Clear 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15506	Reacti-Bind™ Streptavidin Coated Plates (HBC), Black 384-Well Plates with SuperBlock® Blocking Buffer	5 plates
15505	Reacti-Bind™ Streptavidin Coated Plates (HBC), White 384-Well Plates with SuperBlock® Blocking Buffer	5 plates

Affinity Purification of Antibodies

Antibodies specific for an antigen of interest are one of the most useful and important tools that biology researchers can possess. The production and use of specific antibodies as detection probes and purification ligands (i.e., immunotechnology) has revolutionized bioresearch and diagnostic technologies. Animals immunized with prepared antigens will produce specific antibodies against the antigen. When purified from serum or hybridoma cell lines prepared from tissue of the immunized animal, the antibody may be used directly (or after labeling with enzyme or fluorescent tags) to probe the specific antigen in Western blotting, ELISA or a variety of other applications. Antibodies are most commonly purified by one of two affinity purification methods: general immunoglobulin purification or specific antibody purification.

General Purification of Immunoglobulins

Because antibodies have predictable structure, including relatively invariant domains, it has been possible to identify certain protein ligands that are capable of binding generally to antibodies, regardless of the antibody's specificity to antigen. Protein A, Protein G and Protein L are three bacterial proteins whose antibody-binding properties have been well characterized. These proteins have been produced recombinantly and used routinely for affinity purification of key antibody types from a variety of species. A genetically engineered recombinant form of Protein A and G, called Protein A/G, is also available. These antibody-binding proteins are available immobilized to agarose gel, UltraLink® Biosupport Medium and coated onto microplates (see previous section on Solid Supports for Affinity Purification, page 6).

Proteins A, G, A/G and L bind to antibodies at sites other than the antigen-binding domain. Therefore, these proteins can be used in purification schemes such as immunoprecipitation (see discussion of Immunoprecipitation that follows).

Proteins A, G, A/G and L have unique properties, which make each one suitable for different types of antibody targets (e.g., antibody subclass or animal species). It is important to realize that use of Protein A, G or L results in purification of general immunoglobulin from a crude sample. Depending on the sample source, antigen-specific antibody may account for only a small portion of the total immunoglobulin in the sample. For example, generally only 2-5% of total IgG in mouse serum is specific for the antigen used to immunize the animal.

ImmunoPure® and UltraLink® Immobilized Protein L, Protein A, Protein G and Protein A/G

Protein A, Protein G, Protein A/G and Protein L are native or recombinant proteins of microbial origin with the ability to bind to mammalian immunoglobulin molecules. Pierce's highly purified form of these proteins is covalently coupled to an agarose support to provide an efficient method of purifying antibodies. The "Plus" format of these affinity supports has twice the amount of protein immobilized per ml of gel. Pierce supplies Protein A, Protein G and Protein A/G immobilized on UltraLink® Biosupport Medium. The UltraLink® Biosupport has minimal nonspecific binding and is durable. It is a perfect support for working with large volumes of samples requiring fast-flow techniques and large-scale purifications.

The interaction between the various proteins and IgG is not equivalent for all species or all antibody subclasses. The table below will help you decide which affinity support is best for your application.

	Recombinant Protein L	**Native Protein A**	**Recombinant Protein A**	**Recombinant Protein G**	**Recombinant Protein A/G**
Source	*Peptostreptococci*	*Staphylococcus aureus*	*Bacillus*	*Streptococci*	*Bacillus*
Molecular Weight	35,800	42,000	44,600	22,000	50,449
Number of Binding Sites for IgG	4	4	5	2	4
Albumin-Binding Site	No	No	No	No	No
Optimal Binding pH	7.5	8.2	8.2	5	5-8.2
Binds to	V_LK	Fc	Fc	Fc	Fc

Table 13. Binding Characteristics of Immunoglobulin Binding Proteins and Thiophilic Gel*

Antibody	Protein A	Protein G	Protein A/G	Protein L**	T-Gel™ Adsorbent
Human IgG	s	s	s	s	m
Mouse IgG	s	s	s	s	s
Rabbit IgG	s	s	s	w	m
Goat IgG	w	s	s	nb	s
Rat IgG	w	m	m	s	s
Sheep IgG	w	s	s	nb	s
Cow IgG	w	s	s	nb	s
Guinea Pig IgG	s	w	s	–	s
Hamster IgG	m	m	m	s	–
Pig IgG	s	w	s	s	s
Horse IgG	w	s	s	–	s
Donkey IgG	m	s	s	–	–
Dog IgG	s	w	s	–	s
Cat IgG	s	w	s	–	s
Monkey IgG (Rhesus)	s	s	s	–	s
Chicken IgY (IgG)	nb	nb	nb	nb	m
Human IgM	w	nb	w	s	m
Human IgE	m	nb	m	s	–
Human IgD	nb	nb	nb	s	–
Human IgA	w	nb	w	s	m
Human IgA_1	w	nb	w	s	m
Human IgA_2	w	nb	w	s	m
Human IgG_1	s	s	s	s	m
Human IgG_2	s	s	s	s	m
Human IgG_3	w	s	s	s	m
Human IgG_4	s	s	s	s	m
Human Fab	w	w	w	s	m
Human ScFv	w	nb	w	s	m
Mouse IgG_1	w	m	m	s	s
Mouse IgG_{2a}	s	s	s	s	s
Mouse IgG_{2b}	s	s	s	s	s
Mouse IgG_3	s	s	s	s	s
Rat IgG_1	w	m	m	s	s
Rat IgG_{2a}	nb	s	s	s	s
Rat IgG_{2b}	nb	w	w	s	s
Rat IgG_{2c}	s	s	s	s	s
Cow IgG_1	w	s	s	nb	s
Cow IgG_2	s	s	s	nb	s
Sheep IgG_1	w	s	s	nb	s
Sheep IgG_2	s	s	s	nb	s
Goat IgG_1	w	s	s	nb	s
Goat IgG_2	s	s	s	nb	s
Horse IgG(ab)	w	nb	w	–	s
Horse IgG(c)	w	nb	w	–	s
Horse IgG(T)	nb	s	s	–	s
Mouse IgM	nb	nb	nb	s	m

w = weak binding, m = medium binding, s = strong binding, nb = no binding, – means information not available

** Data represent a summary of binding properties reported in the literature. Inevitably some discrepancies exist among reported values as a result of differences in binding buffer conditions and form of the proteins used.*

***Binding will occur only if the appropriate kappa light chains are present. Antibodies lambda light chains will not bind, regardless of their class and subclass.*

Protein A

Protein A Characteristics and IgG Binding Properties

Protein A is a cell wall component produced by several strains of *Staphylococcus aureus*. It consists of a single polypeptide chain (MW 42,000) and contains little or no carbohydrate.[1] Protein A binds specifically to the Fc region of immunoglobulin molecules, especially IgG. It has four high-affinity (K_a = 10^8 l/mole) binding sites that are capable of interacting with the Fc region of IgGs of several species.[2] The molecule is heat-stable and retains its native conformation even after exposure to denaturing reagents such as 4 M urea, 4 M thiocyanate and 6 M guanidine hydrochloride.[3]

In its immobilized form (e.g., covalently coupled to beaded agarose gel), Protein A has been used extensively for isolation of a wide variety of immunoglobulins from several species of mammals. However, the interaction between Protein A and IgG is not equivalent for all animal sources and subclasses of IgG. For example, human IgG_1, IgG_2 and IgG_4 bind strongly to Protein A, while IgG_3 does not bind.[2] In mice, IgG_{2a} , IgG_{2b} and IgG_3 bind strongly to Protein A, but IgG_1 (the dominant subclass in serum) binds only weakly using standard buffer conditions. Most rat IgG subclasses bind weakly or not at all to Protein A. Despite this variability, Protein A is very effective for routine affinity purification of IgG from the serum of many species. It is especially suited for purification of polyclonal antibodies from rabbits.

Weak binding of Protein A to mouse IgG_1 using traditional Tris•HCl or sodium phosphate buffer systems is of particular concern and is one reason to choose Protein G when purifying mouse antibodies. However, Pierce has developed a binding buffer that allows Protein A to bind mouse IgG_1 nearly as well as other subclasses (see subsequent discussion of IgG Binding and Elution Buffers).

The variable binding properties of Protein A for different subclasses of IgG can be used advantageously to separate one IgG type from another. Antibodies that do not bind to immobilized Protein A may be recovered by collecting the non-bound ("flow-through") fractions during binding and wash steps in an affinity purification procedure. In this way, human IgG_3 and other immunoglobulin subclasses may be isolated from those that do bind to Protein A; however, other IgGs and serum proteins, such as albumin, will also be present in the non-bound fraction. Certain IgM, IgD and IgA molecules also do not bind to Protein A and may be separated from Protein A-binding proteins in the same manner.

Immobilized Protein A Products

Pierce offers Protein A immobilized to several different solid supports and made available in different binding capacity formats, package sizes and kit formats. ImmunoPure® Immobilized Protein A generally denotes those products using highly purified Protein A that is covalently coupled to 6% cross-linked beaded agarose gel. ImmunoPure® Immobilized Protein A has a binding capacity of 12-19 mg of human IgG per ml of gel. It exhibits excellent elution properties when used with Pierce buffer systems (see Figure), which generally enable the gel to be regenerated and used for at least 10 rounds of purification. Supplied as a 50% gel slurry in storage buffer, ImmunoPure® Immobilized Protein A is the usual choice either for small-scale batch method purification procedures or for packing gravity-flow columns.

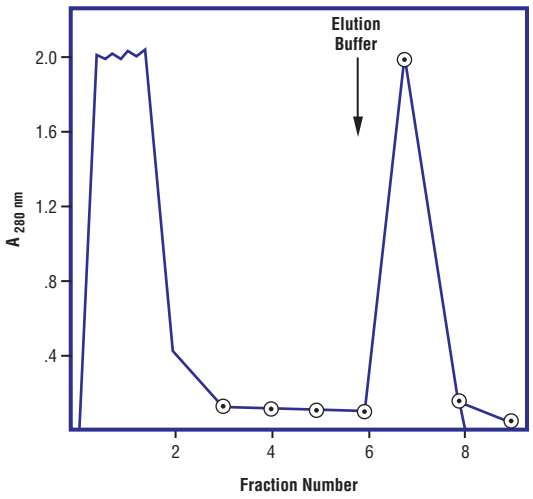
Protein A AffinityPak™ Columns (Product # 20356) are 5 x 1 ml pre-packed plastic columns of ImmunoPure® Immobilized Protein A. The stop-flow action of AffinityPak™ Columns prevents the gel bed from drying out when a column is left unattended for short periods of time.

ImmunoPure® Immobilized Protein A is also available on Trisacryl® GF-2000, rather than agarose gel. This stable affinity support can withstand the high-throughput volumes required in large-scale purification procedures. In addition, because Trisacryl® GF-2000 is a hydrophilic matrix, nonspecific binding of proteins is minimized.

UltraLink® Immobilized Protein A is another alternative for large-scale, high-throughput applications. UltraLink® Biosupport Medium is composed of a hydrophilic, cross-linked *bis*-acrylamide/azlactone copolymer. It has an average bead diameter of 60 µm, can withstand pressures exceeding 100 psi, retains good chromatographic properties using flow rates up to 3,000 cm/hour and displays extremely low nonspecific binding. UltraLink® Immobilized Protein A is the ideal choice for medium-pressure liquid chromatographic systems.

Immobilized Recomb® Protein A (Product # 20365, 20366) uses a genetically engineered form of Protein A that is produced recombinantly in a nonpathogenic form of *Bacillus*. Nonessential regions have been removed, and five IgG-binding sites are included, resulting in a mass of 44.6 kDa.Some researchers believe that the recombinant form should be used if the antibody preparation has strict requirements for being enterotoxin-free. Otherwise, the native form serves as a highly efficient means for purifying antibodies. Immobilized Recomb® Protein A is also compatible with ImmunoPure® Binding and Elution Buffers.

For the greatest convenience, choose the ImmunoPure® (A) IgG Purification Kit (Product # 44667). This kit contains everything needed to isolate IgG from rabbit or mouse serum or ascites fluid, as well as other sample types. The included ImmunoPure® Buffer System provides optimal binding and elution of IgG with Immobilized Protein A. The columns in the kit can be regenerated at least 10 times without a significant loss of binding capacity.

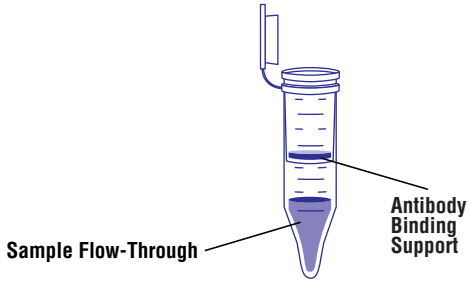


Affinity chromatographic purification of mouse IgG from mouse ascites fluid using Pierce Immobilized Protein A and the ImmunoPure® Buffer System. From 1 ml of mouse ascites fluid, 5.5 mg of mouse IgG was recovered.

To quickly purify small batches of antibody, use NAb™ Spin Purification Kits. These kits are designed to purify up to 1 mg of antibody in about one hour using a simple, benchtop protocol. NAb™ Kits are available with Proteins A, G or L and all of the reagents needed for antibody purification. The resin in each kit can be reused up to 10 times without loss of activity.

Simplified Bench-top Purification Protocol

1. Wash the gel.
2. Incubate antibody-containing sample with Immobilized Protein A, Protein G or Protein L support.
3. Wash away unbound material.
4. Elute the antibody.



Reference

1. Sjoquist, J., *et al.* (1972). *Eur. J. Biochem.* **29**, 572-578.
2. Hjelm, H., *et al.* (1975). *Eur. J. Biochem.* **57**, 395-403.
3. Sjoholm, I., *et al.* (1975). *Eur. J. Biochem.* **51**, 55-61.

Immobilized Protein A

Ordering Information

Product #	Description	Pkg. Size
20333	**ImmunoPure® Immobilized Protein A** Support: Cross-linked 6% beaded agarose Capacity: 12-19 mg human IgG/ml of gel	5 ml
20356	**AffinityPak™ Protein A Columns**	5 x 1 ml
20334	**ImmunoPure® Immobilized Protein A**	25 ml
44667	**ImmunoPure® (A) IgG Purification Kit** *Contains everything needed to isolate IgG from mouse ascites or other serum.* Includes: ImmunoPure® (A) IgG Binding Buffer ImmunoPure® IgG Elution Buffer Protein A AffinityPak™ Columns Excellulose™ Desalting Columns	Kit 1,000 ml 500 ml 5 x 1 ml 5 x 5 ml
20338	**ImmunoPure® Immobilized Protein A** Support: Trisacryl® GF 2000 Capacity: >15 mg human IgG/ml of gel	5 ml
53139	**UltraLink® Immobilized Protein A** Support: UltraLink® Biosupport Medium Capacity: ≥16 mg of human IgG/ml of gel	5 ml

Immobilized Protein A Plus … with twice the amount of Protein A is coupled per ml of gel.

Product #	Description	Pkg. Size
22811	**ImmunoPure® Immobilized Protein A Plus** Support: Cross-linked 6% beaded agarose Capacity: ≥35 mg of human IgG/ml of gel; 16-17 mg mouse IgG/ml of gel	5 ml
22814	**AffinityPak™ Immobilized Protein A Plus Columns**	5 x 1 ml
22812	**ImmunoPure® Protein A Plus**	25 ml
44679	**ImmunoPure® (A) Plus IgG Purification Kit** *Sufficient for isolating 800 mg of mouse IgG.* Includes: Prepacked ImmunoPure® Plus Immobilized Protein A Columns Binding Buffer Elution Buffer Excellulose™ Desalting Columns	Kit 5 x 1 ml 1,000 ml 500 ml 5 x 5 ml
53142	**UltraLink® Immobilized Protein A Plus** Support: UltraLink® Biosupport Medium Capacity: ≥30 mg of human IgG/ml of gel	5 ml
45200	**NAb™ Protein A Spin Purification Kit** Includes: Immobilized Protein A Plus Binding Buffer Elution Buffer Spin X Tubes Microcentifuge tubes	Kit 1 ml 500 ml 50 ml 12 72
15130	**Reacti-Bind™ Protein A Coated 96-Well Plates**	5 plates
15132	**Reacti-Bind™ Protein A Coated Strip Plates**	5 plates

References

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Higashi, I., *et al.* (2000). *Clin. Chem.* **46**, 297-299.

Immobilized Recomb® Protein A

Our recombinant form of immobilized Protein A, manufactured with a leak-resistant linkage.

Highlights:

- Includes disposable column trial kit
- Support: 6% cross-linked agarose beads

References

Bjork, I., *et al.* (1972). *Eur. J. Biochem.* **29,** 579-584.
Goding, J.W. (1978). *J. Immunol. Method* **20,** 241-253.
Lindmark, R., *et al.* (1983). *J. Immunol. Method* **62,** 1-13.
Surolia, A., *et al.* (1982). *Trends Biochem. Sci.* **7,** 74-76.
Kronvall, G., *et al.* (1970). *J. Immunol.* **105,**1116-1123.
Reeves, H.C., *et al.* (1981). *Anal. Biochem.* **115** 194-196.
Kilion, J.J. and Holtgrewe, E.M. (1983). *Clin. Chem.* **29,** 1982-1984.
Ey, P.L., *et al.* (1978). *Immunochemistry* **15,** 429-436.
Bigbee, W.L., *et al.* (1983). *Mol. Immunol.* **20,** 1353-1362.

Ordering Information

Product #	Description	Pkg. Size
20365	**Immobilized Recomb® Protein A** Capacity: ≥12 mg human IgG/ml of gel using the ImmunoPure® (A) IgG Buffer System	5 ml
20366	**Immobilized Recomb® Protein A** Capacity: ≥12 mg human IgG/ml of gel using the ImmunoPure® (A) IgG Buffer System	25 ml

Protein G

Protein G Characteristics and IgG Binding Properties

Protein G is a bacterial cell wall protein isolated from group G streptococci.[1] Like Protein A from *Staphylococcus aureus*, Protein G binds to most mammalian immunoglobulins primarily through their Fc regions. Protein G binds weakly to Fab fragments.[1] Sequencing of DNA that encodes native Protein G indicates that there are two immunoglobulin binding sites, as well as albumin and cell surface binding sites.[2] In the recombinant form of Protein G, these albumin and cell surface binding sites have been eliminated to reduce nonspecific binding when purifying immunoglobulins. With the albumin site removed, recombinant Protein G can be used to separate albumin from crude human immunoglobulin samples. Recombinant Protein G has a mass of approximately 22 kDa. However, its apparent mass by SDS-PAGE is nearly 34 kDa.

Immobilized Protein G is most commonly used for the purification of mammalian monoclonal and polyclonal antibodies that do not bind well to Protein A. It has been reported that most mammalian immunoglobulins bind with greater affinity to Protein G than Protein A.[1] There are, however, species to which Protein A has greater affinity.[3] Protein G binds with significantly greater affinity to several immunoglobulin subclasses including human IgG_3 and rat IgG_{2a}. Unlike Protein A, Protein G does not bind to human IgM, IgD or IgA.[1]

Differences in binding characteristics between Protein A and Protein G are explained by differences in the immunoglobulin binding sites of each protein. Although the tertiary structures of these proteins are similar, their amino acid compositions differ significantly.

Inconsistency in reporting of Protein G binding characteristics occurs in the literature. One cause for this inconsistency likely results from differences in the particular source and isolation method used for the native Protein G characterized in each study. In addition, several methods have been used to assess relative binding affinity including radiolabeling experiments and ELISA techniques, the results of which are not directly comparable. Finally, significant binding differences result from different binding buffers used with Protein G. Optimal binding for most immunoglobulins to Protein G occurs in sodium acetate buffer, pH 5.0,[4] although many studies have used more neutral Tris or phosphate buffers for binding. Approximately 44% more IgG from rat serum bound to Protein G using acetate buffer, pH 5.0 [e.g., ImmunoPure® (G) Binding Buffer, Product # 21011] compared to Tris•HCl pH 7.5 buffer.

Immobilized Protein G Products

Pierce Immobilized Protein G Products incorporate the recombinant form of Protein G immobilized to either 6% cross-linked beaded agarose or UltraLink® Biosupport Medium. For a more detailed description of supports, see the previous pages about Immobilized Protein A Products. Both types of immobilized Protein G utilize coupling chemistries that are leak-resistant and provide a matrix with minimal nonspecific binding. Both supports can be regenerated and reused multiple times when stored properly.

Like Immobilized Protein A already discussed, Immobilized Protein G is offered in several package sizes and kit formats. The ImmunoPure® (G) IgG Purification Kit includes a 2 ml pre-packed column of ImmunoPure® Immobilized Protein G, as well as binding and elution buffers and desalting columns. The 2 ml affinity column will bind 20-30 mg of human IgG when using the included ImmunoPure® Buffers.

References

1. Bjorck, L. and Kronvall, G. (1984). *J. Immunol.* **133,** 969-974.
2. Guss, B., *et al.* (1986). *EMBO J.* **5,** 1567-1575.
3. Eliasson, M., *et al.* (1988). *J. Biol. Chem.* **263,** 4323-4327.
4. Åkerström, B. and Bjorck, L. (1986). *J. Biol. Chem.* **261,** 10240-10247.

Immobilized Protein G Products

Gives better selectivity for IgG isotype than Protein A.

Ordering Information

Product #	Description	Pkg. Size
20398	**ImmunoPure® Immobilized Protein G** Support: Cross-linked 6% beaded agarose Capacity: 11-15 mg human IgG/ml of gel	2 ml
20399	**ImmunoPure® Immobilized Protein G**	10 ml
44441	**ImmunoPure® (G) IgG Purification Kit** Includes: ImmunoPure® Immobilized Protein G Column ImmunoPure® (G) Binding Buffer ImmunoPure® IgG Elution Buffer Excellulose™ Desalting Columns	Kit 1 x 2 ml 240 ml 120 ml 5 x 5 ml
53125	**UltraLink® Immobilized Protein G** Support: UltraLink® Biosupport Medium Capacity: ≥20 mg of human IgG/ml of gel	2 ml
53127	**UltraLink® AffinityPak™ Immobilized Protein G Columns**	2 x 2 ml
53126	**UltraLink® Immobilized Protein G**	10 ml

Immobilized Protein G Plus … with twice the amount of Protein G immobilized per ml of gel.

Product #	Description	Pkg. Size
22851	**ImmunoPure® Immobilized Protein G Plus** Support: Cross-linked 6% beaded agarose Capacity: ≥20 mg human IgG/ml of gel	2 ml
22852	**ImmunoPure® Immobilized Protein G Plus**	10 ml
53128	**UltraLink® Immobilized Protein G Plus** Support: UltraLink® Biosupport Medium Capacity: ≥25 mg of human IgG/ml of gel	2 ml
45201	**NAb™ Protein G Spin Purification Kit** Includes: Immobilized Protein G Plus Binding Buffer Elution Buffer Spin X Tubes Microcentrifuge Tubes	Kit 1 ml 500 ml 50 ml 12 72
15131	**Reacti-Bind™ Protein G Coated 96-Well Plates**	5 plates
15133	**Reacti-Bind™ Protein G Coated Strip Plates**	5 plates

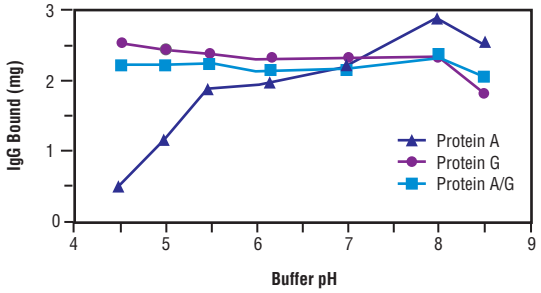
Protein A/G

Protein A/G is a genetically engineered protein that combines the IgG binding profiles of both Protein A and Protein G. Protein A/G is a gene fusion product secreted from a nonpathogenic form of *Bacillus*. Protein A/G (MW 50,449) is designed to contain four Fc binding domains from Protein A and two from Protein G. The secreted protein is readily isolated in a pure form from fermentation medium. Protein A/G is not as pH-dependent as Protein A (Figure) but otherwise has the additive properties of Protein A and G.

Protein A/G binds to all human IgG subclasses. In addition, it binds to IgA, IgE, IgM and, to a lesser extent, IgD. Protein A/G also binds well to all mouse IgG subclasses but does not bind mouse IgA, IgM or serum albumin.[1] This makes Protein A/G an excellent tool for purification and detection of mouse monoclonal antibodies from IgG subclasses, without interference from IgA, IgM and murine serum albumin. Individual subclasses of mouse monoclonals are more likely to have a stronger affinity to the chimeric Protein A/G than to either Protein A or Protein G.[2]

Immobilized Protein A/G is an ideal choice for purification of polyclonal or monoclonal IgG antibodies whose subclasses have not been determined. Overall binding capacity is greater when pH 8.0 buffer (optimal for Protein A) is used rather than pH 5.0 buffer, which is optimal for Protein G used alone. Furthermore, ImmunoPure® (A) Binding Buffer provides for greater binding than Tris•HCl, pH 8.0 (see description of IgG Binding and Elution Buffers on page 40).

Immobilized Protein A/G is offered in similar package sizes and kit formats as Immobilized Protein A and Protein G. ImmunoPure® (A/G) IgG Purification Kit, like the Protein G purification kit, includes a single 2 ml affinity column and accessories.



Comparison of the binding characteristics of mouse IgG at various buffer pH levels.

References
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ImmunoPure® Immobilized Protein A/G and (A/G) IgG Purification Kit

Binds all IgG species that bind both Protein A and Protein G, taking the guesswork out of isolating your antibody.

Ordering Information

Product #	Description	Pkg. Size
20421	**ImmunoPure® Immobilized Protein A/G** *Includes disposable column trial kit.* Support: Cross-linked 6% beaded agarose Capacity: ≥ 7 mg of human IgG/ml gel	3 ml
20422	**ImmunoPure® Immobilized Protein A/G** *Includes disposable column trial kit.*	15 ml
44902	**ImmunoPure® (A/G) IgG Purification Kit** Includes: Immobilized Protein A/G Column ImmunoPure® IgG Binding Buffer ImmunoPure® IgG Elution Buffer Desalting Columns	Kit 1 x 2 ml 240 ml 120 ml 5 x 5 ml

Product #	Description	Pkg. Size
53132	**UltraLink® Immobilized Protein A/G** Support: UltraLink® Biosupport Medium Capacity: ≥ 20 mg of human IgG/ml gel	2 ml
53133	**UltraLink® Immobilized Protein A/G**	10 ml
	Immobilized Protein A/G Plus… with twice the amount of Protein A/G per ml of gel.	
53135	**UltraLink® Immobilized Protein A/G Plus** Support: UltraLink® Biosupport Medium Capacity: ≥ 28 mg human IgG/ml gel	2 ml
15138	**Reacti-Bind™ Protein A/G Coated Strip Plates**	5 plate

Protein L

Protein L is an immunoglobulin-binding protein (MW 35,800) that originates from the bacteria *Peptostreptococcus magnus*, but is now produced recombinantly. Unlike Protein A and Protein G, which bind primarily through Fc regions (i.e., heavy chain) of immunoglobilins, Protein L binds immunoglobulins through interactions with their light chains. Since no part of the heavy chain is involved in the binding interaction, Protein L binds a wider range of Ig classes than Protein A or G. Protein L will bind to representatives of all classes of Ig including IgG, IgM, IgA, IgE, IgD and IgY. Single-chain variable fragments (ScFv) and Fab fragments can also be bound by Protein L.

Despite this wide-ranging binding capability with respect to Ig classes (which are defined by heavy chain type), Protein L is not a universal immunoglobilin-binding protein. Binding of Protein L to immunoglobulins is restricted to those containing kappa light chains (i.e., κ chain of the V_L domain).[1] In humans and mice, kappa (κ) light chains predominate. The remaining immunoglobulins have lambda (λ) light chains. Furthermore, Protein L is effective in binding only certain subtypes of kappa light chains. For example, it binds human VκI, VκIII and VκIV subtypes but does not bind the VκII subtype. Binding of mouse immunoglobulins is restricted to those having VκI light chains.[1]

Given these specific requirements for effective binding, immobilized Protein L is not appropriate for general polyclonal antibody purification from serum, which contains a mixture of immunoglobulins having different types of light chains. The main application for immobilized Protein L is purification of monoclonal antibodies from ascites or culture supernatant that are known to have the kappa light chain.

Protein L is extremely useful for purification of V_Lκ-containing monoclonal antibodies from culture supernatant because it does not bind bovine immunoglobilins, which are present in the media serum supplement. Also, in contrast to Protein A and G, Protein L is very effective at binding IgM. Although it binds to the Fab portion of the immunoglobulin monomer, Protein L does not interfere with the antigen-binding site of the antibody. Therefore, Protein L potentially can be used in immunoprecipitation (IP) procedures.

ImmunoPure® Immobilized Protein L is offered in several formats including gel slurries, pre-packed gravity-flow columns and as column and spin cup kits.

Reference

1. Nilson, B., *et al.* (1992). *J. Biol. Chem.* **267**, 2234-2238.

ImmunoPure® Protein L Products

Purify ScFv or Fab fragments that have kappa light chains.

Highlights:

- Binds to the VL region of kappa light chains (human I, III and IV and Mouse I) without interfering with antigen-binding sites
- Binds to all classes of IgG (e.g., IgG, IgM, IgA, IgE and IgD)
- Does not bind bovine, goat or sheep immunoglobulins
- Binds single-chain variable fragments (ScFv)

Applications:

- Purification/detection of ScFv and Fab fragments containing kappa light chains
- Purification/detection of IgG, IgM, IgA, IgE and IgD
- Purification of monoclonal antibodies from BSA- or FCS-supplemented media because Protein L does not bind bovine antibodies
- Purification/detection of recombinantly produced or engineered antibodies

References

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Åkerström, B. and Björck, L. (1989). *J. Biol. Chem.* **264,** 19740-19746.

Ordering Information

Product #	Description	Pkg. Size
20510	**ImmunoPure® Immobilized Protein L** Capacity: 4-5 mg human IgG/ml of gel	2 ml
20540	**AffinityPak™ Protein L Columns** Capacity: 4-5 mg human IgG/ml of gel	2 x 2 ml
20520	**ImmunoPure® Immobilized Protein L Plus** Capacity: 8-10 mg human IgG/ml of gel	2 ml
20550	**ImmunoPure® (L) Immunoglobulin Purification Kit** Includes: AffinityPak™ Immobilized Protein L Column Binding Buffer Elution Buffer Excellulose™ Desalting Columns	Kit 1 x 2 ml 500 ml dry pack 120 ml 5 x 5 ml
20530	**NAb™ Protein L Spin Purification Kit** Capacity: 8-10 mg human IgG/ml of gel Includes: Immobilized Protein L Plus Binding Buffer Elution Buffer Spin X Tubes Microcentrifuge Tubes	Kit 1 ml 500 ml dry pack 50 ml 12 72
15190	**Reacti-Bind™ Protein L Coated 96-Well Plates**	5 plates

IgG Binding and Elution Buffers for Protein A, G, A/G and L

Binding and Elution Steps in Affinity Purification

Affinity purification procedures involving interaction of an antibody with its antigen generally use binding buffers at physiologic pH and ionic strength. However, many antibody purification methods do not use the antibody-antigen interaction; rather, they involve binding of antibodies by immobilized ligands that are not the antigen. In such cases, optimal binding conditions are determined by the unique properties of the antibody-ligand interaction, which may be different from physiologic pH and ionic strength.

Once the binding interaction occurs (i.e., the antibody is "captured" by the immobilized ligand), the support is washed with additional buffer to remove nonbound components of the sample. Finally, elution buffer is added to break the binding interaction and release the target molecule, which is then collected in its purified form. Elution buffer can dissociate binding partners by extremes of pH (low or high), high salt (ionic strength), the use of detergents or chaotropic agents that denature one or both of the molecules, removal of a binding factor, or competition with a counter ligand. In most cases, subsequent dialysis or desalting is required to exchange the purified protein from elution buffer into a more suitable buffer for storage or use.

ImmunoPure® IgG Binding and Elution Buffers have been optimized to provide the highest possible efficiency of IgG binding and elution using immobilized Protein A, Protein G and Protein A/G. Use of other buffer formulations may significantly alter not only the binding capacity but also the volumes of wash buffer required to ensure good purification.

General Binding and Elution Buffers for Protein A, G, A/G and L

Although Protein A, G and A/G bind immunoglobulins adequately at physiologic pH and ionic strength (as with phosphate buffered saline, pH 7.2), optimal binding conditions are different for each protein. For this reason, Pierce offers separate ImmunoPure® IgG Binding Buffers for use with the immobilized "alphabet protein" products. All ImmunoPure® Buffers have long shelf lives and are premixed for maximum ease of use.

The ImmunoPure® (A) IgG Binding Buffer is a unique, phosphate-based formulation (pH 8.0) developed by Pierce scientists to achieve maximum binding capacity of IgG to immobilized Protein A. Overall IgG binding capacity is increased with this buffer relative to traditional binding buffers (Table 14). Most notably, the otherwise weak binding of mouse IgG_1 is greatly improved.

ImmunoPure® (G) IgG Binding Buffer uses sodium acetate (pH 5.0) to obtain the highest possible binding capacity of IgG to immobilized Protein G. The binding buffer for Protein A/G is similar to the ImmunoPure® IgG Binding Buffer for Protein A. The optimal binding with Protein L occurs at pH 7.5; ImmunoPure® Protein L Kits use phosphate buffered saline (PBS) as the binding buffer.

Generally, an ImmunoPure® Binding Buffer is used by combining it 1:1 (v/v) with clarified serum or ascites fluid. For a dilute sample, or to minimize its total volume, a sample can be dialyzed into the recommended buffer. Purity of the immunoglobulin samples will affect the total binding capacity of Protein A, G and A/G; total immunoglobulin binding capacities are higher for purified and concentrated immunoglobulins than for crude serum or dilute samples.

Elution of immunoglobulins that are bound to immobilized alphabet proteins, regardless of the binding buffer used, is most generally accomplished using 0.1 M glycine•HCl (pH 2-3) or other low pH buffer. In the vast majority of cases, this condition breaks affinity interactions without damaging either the immobilized protein (allowing the affinity column to be re-used) or the antibody. ImmunoPure® IgG Elution Buffer uses this acidic (pH 2.8) condition. With this buffer, elution of IgG is usually sharp and complete. For example, nearly all bound IgG will elute in 3 ml of buffer from a 1 ml column of Protein A.

Although brief exposure of antibody to acidic elution buffer usually is not harmful, it is advisable to neutralize the eluate as soon as possible after its recovery to minimize the possibility of degradation. ImmunoPure® IgG Elution Buffer can be neutralized easily by adding 1/10th volume of 1 M Tris•HCl, pH 7.5-9.0. Although long-term storage of the purified antibody in the neutralized buffer may be possible in certain cases, it is common practice to dialyze or desalt into a buffer that is known to be suitable for storage.

Table 14. Binding Capacities with Different Buffers Expressed as mg of IgG Bound per 2 ml of Gel

	Immobilized Protein A		Immobilized Protein G		Immobilized Protein A/G	
Serum Sample	0.1 M Tris•HCl pH 8.0	ImmunoPure® (A) Binding Buffer	0.1 M Tris•HCl pH 8.0	ImmunoPure® (G) Binding Buffer	0.1 M Tris•HCl pH 8.0	ImmunoPure® (A) Binding Buffer
Rabbit	17.81	33.19	21.51	27.75	13.89	19.61
Sheep	2.15	10.64	25.53	33.33	9.83	15.71
Bovine	6.16	22.76	31.72	48.10	15.13	22.06
Mouse	5.25	7.15	5.65	15.05	4.32	11.49
Rat	4.99	8.30	8.43	11.80	5.20	6.66
Horse	6.25	16.50	36.19	21.46	14.88	17.12
Dog	35.77	22.27	13.38	20.55	21.96	24.60
Chicken	0.91	1.21	1.63	7.27	1.21	4.10
Pig	29.61	24.83	21.25	27.51	19.24	29.48
Human	19.88	25.53	11.68	23.59	9.92	17.67

Gentle Ag/Ab Elution Buffer

Some antibodies are extremely labile and irreversibly denature in the acidic conditions of the default ImmunoPure® IgG Elution Buffer. For such situations, Pierce offers ImmunoPure® Gentle Ag/Ab Elution Buffer. This near-neutral (pH 6.55) buffer dissociates affinity-bound immunoglobulins by ionic strength rather than by low pH. While being much less likely to degrade an antibody, it still retains excellent elution properties.

Pierce researchers have tested the effect of exposure to Gentle Elution Buffer on monoclonal antibody activity. In one experiment, three mouse monoclonals were incubated overnight in the Gentle Elution Buffer and then desalted. When analyzed in an ELISA system, all three monoclonals retained full antigen binding capability as compared to untreated controls.

The Gentle Elution Buffer does not require neutralization and is directly compatible with borate, citrate and acetate buffers including ImmunoPure® (G) IgG Binding Buffer. However, Gentle Elution Buffer is not directly compatible with phosphate-containing buffers including ImmunoPure® (A) IgG Binding Buffer, with which it will form an insoluble precipitate. For this reason, ImmunoPure® Gentle Ag/Ab Binding Buffer, pH 8.0 is offered as a substitute for use with Protein A.

Mouse IgG_1 Mild Elution Buffer

A unique opportunity exists in Protein A with its weaker binding affinity to mouse IgG_1 compared to other mouse IgG subclasses. After binding total mouse IgG to immobilized Protein A using ImmunoPure® (A) IgG Binding Buffer, ImmunoPure® Mouse IgG_1 Mild Elution Buffer can be used to selectively elute IgG_1 without affecting the bound state of other IgG subclasses.

The buffer has a mild pH (6.0-6.1) to retain better biological activity in both the recovered antibody and the immobilized Protein A. Neutralization or desalting of the collected IgG_1 is not necessary to retain activity. This advantage is especially important when isolating potentially fragile monoclonal IgG_1 antibodies. Because the majority of mouse monoclonals are of the IgG_1 subclass, this buffer has many applications in the production of monoclonal antibodies.

After eluting the IgG_1, other bound IgGs can be eluted using standard IgG Elution Buffer. ImmunoPure® (A) Binding Buffer and both IgG and IgG_1 Mild Elution Buffers are available as a kit. The system enables quick, clean and mild isolation of mouse IgG_1 from serum, ascites or hybridoma culture supernatant.

Ordering Information

Product #	Description	Highlights	Pkg. Size
54200	ImmunoPure® (A/G) IgG Binding Buffer	• Assures maximum recovery of IgG from immobilized Protein A/G	240 ml
21001 21007	ImmunoPure® (A) IgG Binding Buffer	• High-yield isolation of Mouse IgG_1 using Protein A columns • Premixed and easy to use	1 liter 3.75 liters
21011	ImmunoPure® (G) IgG Binding Buffer	• Assures maximum recovery of IgG from immobilized Protein G	3.75 liters
21004 21009	ImmunoPure® IgG Elution Buffer	• High-yield isolation of IgG from Immobilized Protein A and Protein G	1 liter 3.75 liters
21020 21012	ImmunoPure® Gentle Ag/Ab Binding Buffer, pH 8.0	• Specially formulated and prefiltered • Eliminates use of harsh acidic elution conditions	1.0 liter 3.75 liters
21030 21027 21013	ImmunoPure® Gentle Ag/Ab Elution Buffer, pH 6.6	• Specially formulated for neutral elutions • Not compatible with phosphate buffers	100 ml 500 ml 3.75 liters
21016	ImmunoPure® IgM Binding Buffer	• Specially formulated for optimal binding of mouse IgM	800 ml
21017	ImmunoPure® IgM Elution Buffer	• Specially formulated for optimal recovery of mouse IgM	500 ml
21018	ImmunoPure® MBP Column Preparation Buffer	• Specially formulated for use with Immobilized MBP and IgM Purification Kit	50 ml
21034	Mouse IgG_1 Mild Elution Buffer	• Separate IgG_1 from other IgG subclasses	500 ml
21033	Mouse IgG_1 Mild Binding and Elution Buffer Kit Includes: ImmunoPure® (A) IgG Binding Buffer Mouse IgG_1 Mild Elution Buffer ImmunoPure® IgG Elution Buffer	• Complete kit to allow mouse IgG_1 to be separated from other mouse IgG subclasses	Kit 1 liter 500 ml 1 liter

Thiophilic Gel Antibody Purification

Thiophilic Adsorption

Thiophilic adsorption is a low-cost, efficient alternative to ammonium sulfate precipitation for immunoglobulin purification from crude samples. Ammonium sulfate precipitation must be followed by several additional steps to completely remove contaminants in crude samples. Thiophilic adsorption is a simple, rapid, one-step method for antibody purification from serum, ascites or tissue culture supernatant.

Thiophilic adsorption is a highly selective type of lyotropic salt-promoted protein:ligand interaction phenomenon that has been studied extensively by Porath and co-workers and other researchers.[1] This interaction is termed thiophilic because it is distinguished by proteins that recognize a sulfone group in close proximity to a thioether. Thiophilic adsorption incorporates properties of both hydrophobic and hydrophilic adsorption. However, in contrast to strictly hydrophobic systems, thiophilic adsorption is not strongly promoted by high concentrations of sodium chloride. Instead, thiophilic adsorption is promoted by increased concentrations of water-interacting, non-chaotropic salts such as potassium and ammonium sulfate.

T-Gel™ Adsorbent

T-Gel™ Adsorbent is 6% beaded agarose gel modified to contain simple sulfone/ thioether groups (see Figure). T-Gel™ Adsorbent has a high binding capacity (20 mg of immunoglobulin per ml of gel) and broad specificity toward immunoglobulins derived from various animal species. Notably, thiophilic adsorption is one of few methods available for purification of IgY from chicken (see also subsequent discussion of IgY purification). Among human serum proteins, immunoglobulins and α_2-macroglobulins are preferentially bound by T-Gel™ Adsorbent.[2]

Purification using T-Gel™ Adsorbent results in good protein recovery with excellent preservation of antibody activity. Sample preparation requires the addition of 0.5 M potassium sulfate to the serum, ascites or culture fluid. Greater specificity for immunoglobulins is obtained if the sample is buffered at pH 8.0.

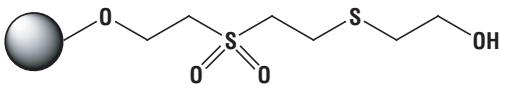
The gentle elution conditions (e.g., 50 mM sodium phosphate, pH 7-8) yield concentrated, essentially salt-free, highly purified immunoglobulins at near neutral pH.

After use, T-Gel™ Adsorbent can be regenerated by treatment with guanidine•HCl. Pierce data indicate that the T-Gel™ Adsorbent column can be used at least 10 times without significant loss of binding capacity.

T-Gel™ Purification Kit includes 4 x 3 ml prepacked columns of T-Gel™ Adsorbent, binding and elution buffers, column storage buffer, and guanidine•HCl for use in column regeneration. This simple, one-step method eliminates the need for post-treatment of the sample before storage or subsequent conjugation to enzymes for use in immunoassays.

Suggested applications for T-Gel™ Adsorbent:

- Efficient and selective isolation of immunoglobulins from human serum under mild conditions[1]
- Convenient and fast method for purification of mouse monoclonals from the culture media of cloned cells or from ascites fluid[2]
- Selective removal of immunoglobulins from fetal calf serum – useful for cell culture in monoclonal antibody production[3]
- Rapid, straightforward procedure yielding essentially pure immunoglobulins from crude rabbit serum[4]
- Purification of IgY from chicken[5]
- Large-scale purification for biotechnology applications



Structure of T-Gel™ Adsorbent.

References

1. Porath, J., *et al.* (1985). *FEBS Lett.* **185**, 306-310.
2. Belew, M., *et al.* (1987). *J. Immunol. Method* **102**, 173-182.
3. Hutchens, T.W. and Porath, J. (1987). *Biochemistry* **26**, 7199-7204.
4. Lihme, A. and Heegaard, P.M.H. (1990). *Anal. Biochem.* **192**, 64-69.
5. Unpublished internal Pierce documents.

T-Gel™ Adsorbent and T-Gel™ Purification Kit

Economical purification of mouse antibodies from ascites fluid.

Table 15. Binding Characteristics of T-Gel™ Adsorbent

Species	Total A_{280} Bound from 1 ml serum	% Purity by HPLC
Human	4.8	70
Mouse	8.6	63
Mouse IgG_1	11.6	92
Mouse IgG_{2a}	9.3	88
Mouse IgG_{2b}	9.8	97
Mouse IgG_3	10.7	94
Rat	13.0	79
Bovine	17.9	90
Calf	11.1	89
Chicken	5.2	76
Dog	12.2	91
Goat	17.3	92
Guinea Pig	11.1	71
Horse	13.0	93
Pig	21.1	90
Rabbit	6.7	84
Sheep	12.3	89

Highlights:

- Binds to Fab and $F(ab)_2$ fragments
- Binds to ScFv[1]
- High-capacity (20 mg/ml), good protein recovery and retention of antibody function
- Broad specificity toward immunoglobulins derived from various animal species (Table 15)
- Binds chicken IgY (also called IgG)
- Simple, rapid, one-step purification for monoclonal antibodies from ascites; easy to scale up
- Used to enrich the immunoglobulin fraction from serum or tissue culture supernatant
- Efficient alternative to ammonium sulfate precipitation for enriching antibodies from crude samples
- Gentle elution conditions yield concentrated, salt-free immunoglobulin at near neutral pH
- High degree of purity

References

1. Schulze, R.A., *et al.* (1994). *Anal. Biochem.* **220**, 212-214.
Palmer, D.A., *et al.* (1994). *Anal. Biochem.* **222**, 281-283.
Porath, J., *et al.* (1985). *FEBS Lett.* **185**, 306-310.
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Belew, M., *et al.* (1987). *J. Immunol. Method* **102**, 173-182.
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Nopper, B., *et al.* (1989). *Anal. Biochem.* **180**, 66-71.
Harsay, E. and Schekman, R. (2002). *J. Cell Biol.* **156(2)**, 271-85.
Koustova, E. *et al.* (2001). *J. Clin. Invest.* **107(6)**, 737-44.
Suh, J.S., *et al.* (1998). *Blood.* **91(3)**, 916-22.

Ordering Information

Product #	Description	Pkg. Size
20500	**T-Gel™ Adsorbent**	10 ml
44916	**T-Gel™ Purification Kit***	Kit
	Includes: T-Gel™ Adsorbent Prepacked Columns	4 x 3 ml
	T-Gel™ Binding Buffer	1,000 ml
	T-Gel™ Elution Buffer	1,000 ml
	T-Gel™ Column Storage Buffer (2X)	100 ml
	Guanidine•HCl Crystals	230 g
	Column Extenders	

IgM Purification

Structure of IgM

IgM is a high molecular weight glycoprotein (MW 900,000-950,000) with a carbohydrate content of approximately 12%. This antibody is found at concentrations of 0.5-2 mg/ml in serum.[1] *In vivo*, IgM has a half life of five days, and its catabolism is two- to three-fold greater than that of IgG.

In the sera of mammals, birds and reptiles, IgM has a pentameric structure. However, mouse and human IgM structure differs in the location of disulfide bridges that link monomers together to form the pentamer (Figure).[2] Disulfides are arranged in series in mouse IgM and in parallel in human IgM.

Challenges to IgM Purification

Protein A binds IgM poorly, in part because binding sites on the Fc region of the monomers are sterically hindered by the pentameric structure of IgM. Until recently, no readily available affinity chromatography product existed for one-step IgM purification. Standard methods for IgM purification generally are multistep, tedious processes or they are not effective for removing all of the major impurities present in IgM samples.[3]

Traditionally, IgM was purified by ammonium sulfate precipitation followed by gel filtration chromatography, ion exchange chromatography or zone electrophoresis.[4] Other methods that have been used include use of DEAE cellulose,[5] immobilized DNA[6] and a combination of ammonium sulfate precipitation and subsequent removal of IgG with Protein A or G.[3]

Nethery, *et al.* developed an IgM affinity purification method using C1q, a 439 kDa complement component that recognizes carbohydrate on cell surfaces.[7] This temperature-dependent binding method yielded relatively pure IgM. However, co-purification of IgG was a problem, and C1q is expensive and difficult to purify.

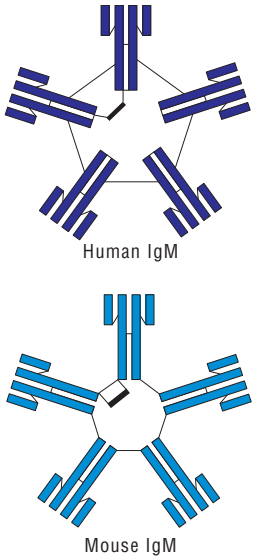
Immobilized Mannan Binding Protein

To develop an effective affinity matrix, Pierce scientists examined C1q and another similarly structured protein, mannan binding protein (MBP). Serum MBP, like C1q, is capable of initiating carbohydrate-mediated complement activation. MBP is a mannose and *N*-acetylglucosamine specific lectin found in mammalian sera, and it has considerable structural homology to C1q.[8] MBP subunits are identical, each with molecular mass of approximately 31 kDa (C1q has six each of three different polypeptide subunits of molecular mass 24-28 kDa). Studies in Pierce labs show that MBP does not bind $F(ab')_2$ and Fab.

Pierce has developed an easy-to-use ImmunoPure® Immobilized Mannan Binding Protein and buffer system to purify IgM. It is most effective for purifying mouse IgM from ascites. Purified IgM can be obtained from a single pass over the affinity column. Human IgM will bind to the support, albeit with slightly lower capacity, and yield a product at least 88% pure as assessed by HPLC. The purification of IgM from other species and mouse serum has not yet been optimized.

IgM purification with ImmunoPure® Immobilized Mannan Binding Protein is temperature- and calcium-dependent. Binding and washing steps are performed at 4°C in 10 mM Tris•HCl (pH 7.4) buffer containing sodium chloride and 20 mM calcium chloride. Elution is made at room temperature in a similar Tris buffer, except that it contains EDTA and is devoid of calcium chloride. An Immobilized MBP Column can be regenerated at least 10 times with no apparent loss of binding capacity.

Immobilized MBP is available in both beaded agarose and UltraLink® Biosupport Medium formats. Binding, elution and column preparation buffers are also available. The ImmunoPure® IgM Purification Kit contains sufficient buffers to perform 10 purifications using a 5 ml column of ImmunoPure® Immobilized MBP. The kit is easy to use and yields 90% pure mouse IgM (from ascites) with a very simple protocol.



Structure of IgM, adapted from Matthew and Reichardt.[9]

References

1. Metzger, H. (1970). *Advanced Immunol.* **121**, 57-113.
2. Milstein, C., *et al.* (1975). *Biochem. J.* **151**, 615-624.
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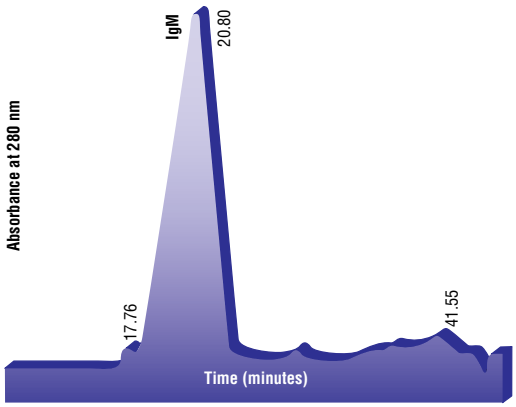
5. Cambier, J. and Butler F. (1974). *Prep. Biochem.* **4(1)**, 31-46.
6. Abdullah, M., *et al.* (1985). *J. Chromatogr.* **347**, 129-136.
7. Nethery, A., *et al.* (1990). *J. Immunol. Method* **126**, 57-60.
8. Ohta, M., *et al.* (1990). *J. Biol. Chem.* **264**, 1980-1984.
9. Matthew, W. and Reichardt, L. (1982). *J. Immunol. Method* **50**, 239-253.

ImmunoPure® Immobilized MBP and IgM Purification Kit

Easy IgM purification … with guaranteed 88% pure mouse IgM!

Demonstration of the High Purity of MBP-purified IgM From Mouse Ascites



The bound material from mouse ascites was eluted from the 5 ml MBP column as described in the Standard Protocol. The highest 280 nm absorbing fraction from the elution was chromatographed using the conditions described in the instruction booklet.

References

Nethery, A., *et al.* (1990). *J. Immunol. Method* **126**, 57-60.
Ohta, M., *et al.* (1990). *J. Biol. Chem.* **265**, 1980-1984.
Nevens, J.R., *et al.* (1992). *J. Chromatogr.* **597**, 247-256.

Ordering Information

Product #	Description	Pkg. Size
22212	**ImmunoPure® Immobilized Mannan Binding Protein** Capacity: ~ 1 mg IgM/ml of gel	10 ml
44897	**ImmunoPure® IgM Purification Kit** Includes: Immobilized MBP Column IgM Binding Buffer IgM Elution Buffer MBP Column Preparation Buffer Column Extender	Kit 5 ml 800 ml 500 ml 50 ml
21016	**ImmunoPure® IgM Binding Buffer**	800 ml
21017	**ImmunoPure® IgM Elution Buffer**	500 ml
21018	**ImmunoPure® MBP Column Preparation Buffer**	50 ml
53123	**UltraLink® Immobilized Mannan Binding Protein** Capacity: > 0.75 mg IgM/ ml of gel	5 ml

Human IgA Purification

Jacalin is an α-D-galactose binding lectin extracted from jack fruitseeds (*Artocarpus integrifolia*). The lectin is a glycoprotein of approximately 40,000 MW composed of four identical subunits. Jacalin immobilized on supports such as agarose has been useful for the purification of human serum or secretory IgA_1. IgA can be separated from human IgG and IgM in human serum or colostrum.[1] IgD is reported to bind to jacalin.[2] Immobilized jacalin is also useful for removing contaminating IgA from IgG samples.

Binding of IgA to immobilized jacalin occurs at physiologic pH and ionic strength, as in phosphate buffered saline (PBS). Elution of bound IgA occurs with competitor ligand (e.g., 0.1 M melibiose or 0.1 M α-D-galactose) in PBS. Pierce offers immobilized jacalin on cross-linked 6% agarose.

References

1. Roque-Barreira, M.C. and Campos-Neto, A. (1985). *J. Immunol. Method* **134(30)**, 1740-1743.
2. Aucouturier, P., *et al.* (1987). *Mol. Immunol.* **24(5)**, 503-511.

Immobilized Jacalin

Ideal for human IgA purification.

Highlights:

- Ideal for preparing human IgA that is free of contaminating IgG
- Found to bind human IgA_1, but **not** human IgA_2 – useful for separating the two subclasses

References

Kumar, G.S., *et al.* (1982). *J. Biosci.* **4**, 257-261.
Roque-Barreira, M.C. and Campos-Neto, A. (1985). *J. Immunol.* **134**, 1740-1743.
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Kondoh, H., *et al.* (1986). *J. Immunol. Method* **88**, 171-173.

Ordering Information

Product #	Description	Pkg. Size
20395	**Immobilized Jacalin** Capacity: 1-3 mg human IgA/ml of gel Support: Cross-linked 6% beaded agarose Loading: 4.5 mg of jacalin/ml of gel	5 ml

Chicken IgY Purification

Properties of IgY

Chickens produce a unique immunoglobulin molecule called IgY. There are several advantages to production and use of IgY over mammalian immunoglobulins. With regard to production, raising and immunizing chickens is relatively simple, chickens are more likely to produce an immune response to conserved mammalian protein antigens, and chickens produce 15-20 times more antibody than rabbits.

Most importantly, IgY is naturally packaged at high concentrations in egg yolks, making repeated collection of antibody from immunized hens noninvasive. A single egg yolk from an immunized chicken contains approximately 300 mg of IgY. Whole eggs or separated egg yolks can be collected and stored frozen for later extraction of antibody.

Other advantages of IgY for use in immunoassays are that it does not bind rheumatoid factor or other anti-mammalian IgGs, does not activate complement, and generally has much lower probability of nonspecific binding to mammalian tissues and extracts.

IgY Purification Methods

One challenge with regard to IgY is that it can be difficult to purify. Protein A, Protein G and other Fc-binding proteins do not bind IgY. However, IgY from chicken possesses light chains that can be bound with high affinity by Protein L.[1-3]

T-Gel™ Adsorbent (see page 42) enables moderate yields of fairly pure IgY from serum and other fluids. However, complete procedures for T-Gel™ Adsorbent have not been developed for use with egg yolks, which have very high lipid concentrations.

Eggcellent® Chicken IgY Purification Kits were specifically developed for efficient purification of IgY from egg yolks. After separating an intact yolk from egg white using an Eggcellent® Egg Separator, Eggcellent® Delipidation Reagent is added to separate the proteins from lipid. The delipidation reagent can also be used to store an egg yolk in the freezer for up to one year. After delipidation, the protein-containing sample fraction is mixed with Eggcellent® IgY Precipitation Reagent to create a relatively pure IgY precipitate that is recovered by centrifugation.

Routinely, 80-120 mg of high purity (>85%), intact IgY can be obtained per egg using the Eggcellent® Kit.

References

1. Bjorch, L. (1988). *J. Immunol.* **140**, 1194-1197.
2. Nilson, B.H.K., *et al.* (1993). *J. Immunol. Method* **164**, 33-40.
3. Akerstrom, B., *et al.* (1989). *J. Biol. Chem.* **264**, 19740-19746.

Eggcellent® Chicken IgY Purification Kit

Purifies 100 mg of chicken IgY with higher purity than ever before!

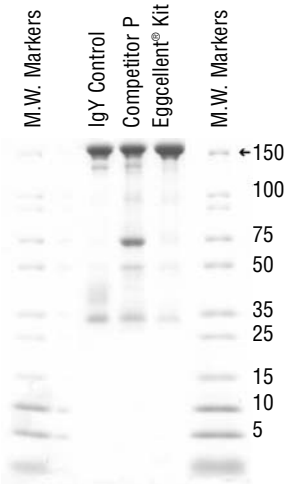
Highlights:

- More for your money – purifies twice the amount of IgY as the leading competitor's kit with a lower cost-per-mg of IgY purified
- Higher purity – 85-95% by SDS-PAGE analysis (see Figure)
- Ease-of-use – the simple precipitation method works without affinity columns
- Flexibility – eggs can be stored in buffer and purified at a later date
- Convenience – use eggs directly out of the refrigerator; no need to wait for them to warm up

Ordering Information

Product #	Description	Pkg. Size
44918	**Eggcellent® Chicken IgY Purification Kit** *Sufficient reagents to purify five egg yolks.* Includes: Eggcellent® Delipidation Reagent Eggcellent® IgY Precipitation Reagent Eggcellent® Egg Separator	Kit 500 ml 500 ml 1
44922	**Eggcellent® Chicken IgY Purification Kit** *Sufficient reagents to purify 25 egg yolks.*	Kit
21055	**Eggcellent® Delipidation Reagent**	500 ml
21057	**Eggcellent® IgY Precipitation Reagent**	500 ml
21060	**Eggcellent® Egg Separator**	1
44916	**T-Gel™ Purification Kit**	Kit

SDS-PAGE Analysis of Eggcellent® Chicken IgY Purification



Chicken IgY was purified according to each manufacturer's instructions. The gel shows the analysis of 2 µg of protein applied per well. The Eggcellent® Kit purified the chicken IgY to a purity level of >85% using GelCode™ Blue Stain Reagent (Product # 24590). The competitor's product achieved only a 53% purity level. The arrow indicates intact IgY.

Affinity Purification of Specific Antibodies

Although Proteins A, G, A/G and L are excellent ligands for purification of total IgG from a sample, purification of an antibody specific for a particular antigen and free of contamination from other immunoglobulins is often required. This can be accomplished by immobilizing the particular antigen used for immunization so that only those antibodies that bind specifically to the antigen are purified in the procedure. Activated affinity supports that can be used to immobilize peptides or other antigens for use in affinity purification are described later in this section.

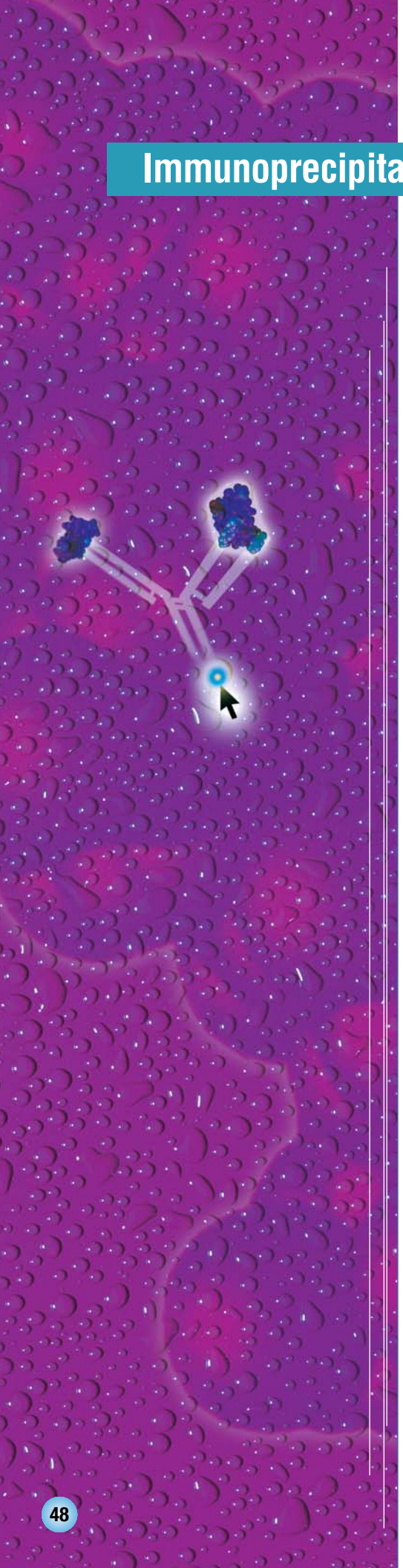
Successful affinity purification of antibody depends on effective presentation of the relevant epitopes on the antigen to binding sites of the antibody. If the antigen is small and immobilized directly to a solid support surface by multiple chemical bonds, important epitopes may be blocked or sterically hindered, prohibiting effective antibody binding. Therefore, it is best to immobilize antigens using a unique functional group (e.g., sulfhydryl on a single terminal cysteine in a peptide) and to use an activated support whose reactive groups occur on spacer arms that are several atoms long. For larger antigens, especially those with multiple sites of immobilization, the spacer arm length becomes less important since the antigen itself serves as an effective spacer between the support matrix and the epitope. Generally, if the antigen was cross-linked to a carrier protein to facilitate antibody production, best results are obtained when the antigen is immobilized for affinity purification using the same chemistry (e.g., reaction to primary amines, sulfhydryls, carboxylic acids or aldehydes). In this way, all epitopes will be available for antibody binding, allowing greater efficiency in purification and recovery of the specific immunoglobulin.

Little variation exists among typical binding and elution conditions for affinity purification of antibodies because at the core of each procedure is the affinity of an antibody for its respective antigen. Since antibodies are designed to recognize and bind antigens tightly under physiologic conditions, most affinity purification procedures use binding conditions that mimic physiologic pH and ionic strength. The most common binding buffers are phosphate buffered saline (PBS) and Tris buffered saline (TBS) at pH 7.2 and 1.5 M NaCl (see pages 4-5 for recipes and convenient premixed buffer packs). Once the antibody has been bound to an immobilized antigen, additional binding buffer is used to wash unbound material from the support. To minimize nonspecific binding, the wash buffer may contain additional salt or detergent to disrupt any weak interactions.

Specific, purified antibodies are eluted from an affinity resin by altering the pH or ionic strength of the buffer (see pages 40-41 for recipes of common elution buffers). Antibodies in general are resilient proteins that tolerate a range of pH from 2.5 to 11.5 with minimal loss of activity, and this is by far the most common elution strategy. In some cases an antibody-antigen interaction is not efficiently disrupted by pH changes or is damaged by the pH, requiring that an alternate strategy be employed.

See section on Covalent Coupling of Affinity Ligands to Solid Supports (page 10) for more information on immobilization of antigens.

Immunoprecipitation and Co-Immunoprecipitation Assays



Immunoprecipitation

Immunoprecipitation (IP) refers to the small-scale affinity purification of antigen using a specific antibody. Classical immunoprecipitation involves the following steps:

1. Incubate specific antibody with an antigen-containing sample.
2. Capture antibody-antigen complex with immobilized Protein A or Protein G agarose gel (Protein A or Protein G binds the antibody, which is bound to its antigen).
3. Wash the gel with buffer to remove non-bound sample components.
4. Elute the antigen (and antibody) by boiling the gel in reducing SDS-PAGE sample buffer.
5. Analyze eluted sample by gel electrophoresis.

Classical IP is usually performed in a microcentrifuge tube with 0.1-1 ml of an antigen-containing sample using 10-50 µl of immobilized Protein A or Protein G gel. The gel is pelleted by centrifugation after each step (washes and elution) and the supernatant is removed. It is practically impossible to identify an elution buffer that will elute antigen from the antibody without also eluting the antibody from Protein A or Protein G. Therefore, the eluted sample will always contain both antigen and antibody, and reducing gel electrophoresis of the eluted sample will yield both antigen bands and heavy- and light-chain antibody fragment bands.

To avoid antibody contamination of the eluted antigen, modifications to the classical IP can be made so that the antibody is permanently immobilized and will not elute with the antigen. One strategy is to first bind the antibody to the Protein A or Protein G gel and then covalently cross-link the antibody to the Protein A or Protein G. Seize® X Immunoprecipitation Kits use this approach. Another strategy is to directly couple antibody to an activated affinity support such as AminoLink® Plus Coupling Gel (see subsequent section on activated affinity supports). Seize® Primary Immunoprecipitation Kits use this approach. Also, because Seize® IP Kits use Handee™ Spin Cup Columns, they improve reproducibility of IP experiments by eliminating resin loss during the washing procedures.

Co-Immunoprecipitation

Immunoprecipitation can be extended to yet another level of affinity purification. In co-immunoprecipitation (co-IP), an antibody is used to capture not only a specific antigen, but also whatever protein or complex is bound to the antigen. When Protein A or Protein G is used to affinity-purify the antibody:antigen complex, there is a minimum of three levels of affinity binding interaction involved in co-IP. For a co-IP to be successful, the binding buffer conditions must be compatible with all levels of binding interaction involved, and the epitope on the antigen must not be blocked by protein:protein interactions.

The complexity of a co-IP system can be reduced by immobilizing the antibody directly and covalently to a resin. This simplifies the binding conditions but, more importantly, it allows the precipitated complex to be eluted from the resin while the antibody remains intact and immobilized. When the precipitated complex is analyzed by SDS-PAGE, or Western blotting, it is free from interfering antibody heavy- and light-chain and the analysis is greatly simplified.

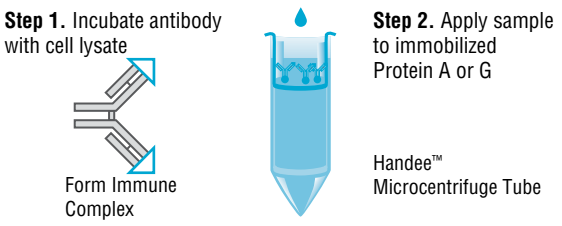
Seize® Classic Immunoprecipitation Kits

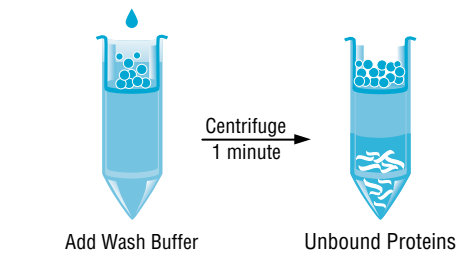
For fast, easy recovery of immune complexed proteins.

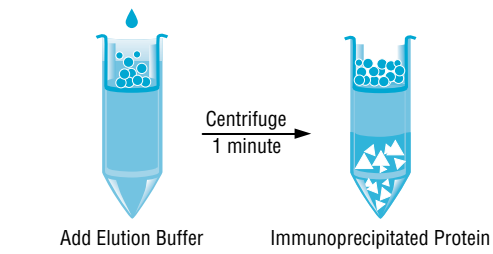
Highlights:

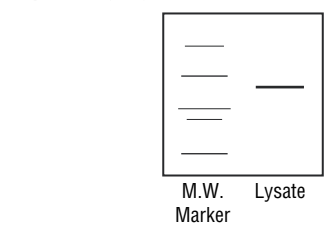
- Improve assay consistency – Handee™ Spin Cups eliminate resin loss
- Easy protocol – immunoprecipitate (IP) out the target protein from crude lysate in just four easy steps
- Fast – IP a protein using Handee™ Spin Cups and tubes in less than one hour
- Economical – reuse the Immobilized Protein A or Protein G support for future IPs
- Complete kits – choose kits with or without cell lysis reagents for bacterial, mammalian or yeast cells

The Seize® Classic Immunoprecipitation Kit Protocol









How it works

Seize® Classic Immunoprecipitation Kits use Handee™ Spin Cups and Microcentrifuge Collection Tubes for easy separation of the solid Protein A or Protein G support from the recovered protein. There is no need to carefully pipette supernatant away from the support. Add the sample containing the immune complex to the Protein A or G support and centrifuge to remove unbound materials. Recover the immunoprecipitated protein by adding elution buffer and then spinning. Next, mix the eluted protein with the sample loading buffer supplied in the kit and analyze by SDS-PAGE.

Ordering Information

Product #	Description	Pkg. Size
45213	**Seize® Classic (A) Immunoprecipitation Kit** *Sufficient reagent for 50 IPs.*	Kit
	Includes: ImmunoPure® Immobilized Protein A Plus	1 ml
	Sample Loading Buffer	5 ml
	Binding Buffer	500 ml
	Elution Buffer	50 ml
	Handee™ Spin Cup Columns	12/pkg.
	Handee™ Microcentrifuge Tubes	72/pkg.
45218	**Seize® Classic (G) Immunoprecipitation Kit** *Sufficient reagent for 50 IPs.*	Kit
	Includes: ImmunoPure® Immobilized Protein G Plus	1 ml
	Sample Loading Buffer	5 ml
	Binding Buffer	500 ml
	Elution Buffer	250 ml
	Handee™ Spin Cup Columns	12/pkg.
	Handee™ Microcentrifuge Tubes	72/pkg.
45217	**Seize® Classic Mammalian Immunoprecipitation Kit** *Sufficient reagent for 50 IPs.*	Kit
	Includes: ImmunoPure® Immobilized Protein G Plus	1 ml
	Sample Loading Buffer	5 ml
	Binding Buffer	500 ml
	Elution Buffer	250 ml
	Handee™ Spin Cup Columns	12/pkg.
	Handee™ Microcentrifuge Tubes	72/pkg.
	M-PER® Mammalian Protein Extraction Reagent	25 ml
69700	**Handee™ Spin Cup Columns**	50 units
69715	**Handee™ Microcentrifuge Tubes, 1.5 ml**	72 tubes

Seize® X Immunoprecipitation Kits

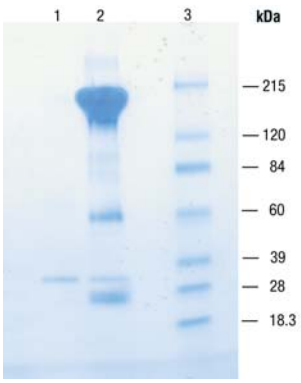
Recover more protein without antibody interference!

Highlights:

- No antibody contamination – antibody heavy and light chains do not appear on SDS-PAGE analysis of precipitated protein
- Improve assay reliability – Handee™ Spin Cup format eliminates resin loss
- More economical – precious primary antibody can be reused to immunoprecipitate more samples
- Complete kits – choose kits with or without cell lysis reagents for bacterial, mammalian or yeast cells
- Can be used with purified or crude antibody preparations

For PAGE analysis without antibody chain interference, the Seize® X Immunoprecipitation Kits offer a better alternative to classic IP.

Seize® X Kits combine cross-linking and affinity chromatography expertise to offer an improved immunoprecipitation method. First, the primary antibody is bound and immobilized to a Protein A or Protein G support using the cross-linking reagent DSS. This properly orients the antibody to "seize" protein from crude cell lysate applied to the immobilized antibody support. Unbound proteins are then centrifuged away and the protein is recovered by using an elution buffer. Analysis on an SDS-PAGE gel shows only a single band for the immunoprecipitated protein without interference from antibody heavy- and light-chain bands. The immobilized antibody support can be reused for future samples.



SDS-PAGE analysis of immunoprecipitation methods. A green fluorescent protein (GFP) fusion protein expressed in *E. coli* was lysed with B-PER® Bacterial Protein Extraction Reagent and immunoprecipitated using a goat anti-GFP antibody. The antibody was either incubated directly with the bacterial lysate using the classical method **(Lane 2)** or immobilized to the Protein G support provided in the Seize® X Bacterial Immunoprecipitation Kit. **(Lane 1)** Immunoprecipitated proteins were reduced, run on an SDS-PAGE gel and stained with GelCode™ Blue Stain Reagent (Product # 24590). **Lane 3** shows BlueRanger® Prestained Protein Molecular Weight Markers (Product # 26681).

References

Seize® X Protein A Immunoprecipitation Kit

Quill, T.A., *et al.* (2001). *Proc Nat. Acad. Sci. USA* **98**, 12527-12531.

Parkin, S.E., *et al.* (2002). *J. Biol. Chem.* **277**, 23563-23572.

Roti, E.C., *et al.* (2002). *J. Biol. Chem.* **277**, 47779-47785.

Seize® X Protein G Immunoprecipitation Kit

Sklyarova, T., *et al.* (2002). *J. Biol. Chem.* **277**, 39840-39849.

Lee, Y.H., *et al.* (papers in press, Nov. 1, 2002). *J. Biol. Chem.*

Kerkela, R., *et al.* (2002). *J. Biol. Chem.* **277**, 13752-13760.

Seize® X Mammalian Immunoprecipitation Kit

Ikemoto, A., *et al.* (2003). *J. Biol. Chem.* **278**, 5929-5940.

The following Seize® X Kits contain sufficient reagents for immobilizing four antibodies and performing 40 IPs.

Ordering Information

Product #	Description	Pkg. Size
45215	**Seize® X Protein A Immunoprecipitation Kit**	Kit
	Includes: ImmunoPure® Immobilized Protein A Plus	1 ml
	Binding/Wash Buffer	500 ml
	Elution Buffer	50 ml
	Sample Loading Buffer	5 ml
	DSS	8 x 2 mg
	Handee™ Spin Cup Columns	12/pkg.
	Handee™ Microcentrifuge Tubes	72/pkg.
45210	**Seize® X Protein G Immunoprecipitation Kit**	Kit
	Includes: ImmunoPure® Immobilized Protein G Plus	1 ml
	Binding/Wash Buffer	500 ml
	Elution Buffer	50 ml
	Sample Loading Buffer	5 ml
	DSS	8 x 2 mg
	Handee™ Spin Cup Columns	12/pkg.
	Handee™ Microcentrifuge Tubes	72/pkg.
45230	**Seize® X Yeast Immunoprecipitation Kit**	Kit
	Includes: ImmunoPure® Immobilized Protein G Plus	1 ml
	Binding/Wash Buffer	500 ml
	Elution Buffer	50 ml
	Sample Loading Buffer	5 ml
	DSS	8 x 2 mg
	Handee™ Spin Cup Columns	12/pkg.
	Handee™ Microcentrifuge Tubes	72/pkg.
	Y-PER® Yeast Protein Extraction Reagent	25 ml
45225	**Seize® X Mammalian Immunoprecipitation Kit**	Kit
	Includes: ImmunoPure® Immobilized Protein G Plus	1 ml
	Binding/Wash Buffer	500 ml
	Elution Buffer	50 ml
	Sample Loading Buffer	5 ml
	DSS	8 x 2 mg
	Handee™ Spin Cup Columns	12/pkg.
	Handee™ Microcentrifuge Tubes	72/pkg.
	M-PER® Mammalian Protein Extraction Reagent	25 ml
45220	**Seize® X Bacterial Immunoprecipitation Kit**	Kit
	Includes: ImmunoPure® Immobilized Protein G Plus	1 ml
	Binding/Wash Buffer	500 ml
	Elution Buffer	50 ml
	Sample Loading Buffer	5 ml
	DSS	8 x 2 mg
	Handee™ Spin Cup Columns	12/pkg.
	Handee™ Microcentrifuge Tubes	72/pkg.
	B-PER® Bacterial Protein Extraction Reagent	25 ml
69700	**Handee™ Spin Cup Columns**	50 units
69715	**Handee™ Microcentrifuge Tubes, 1.5 ml**	72 tubes

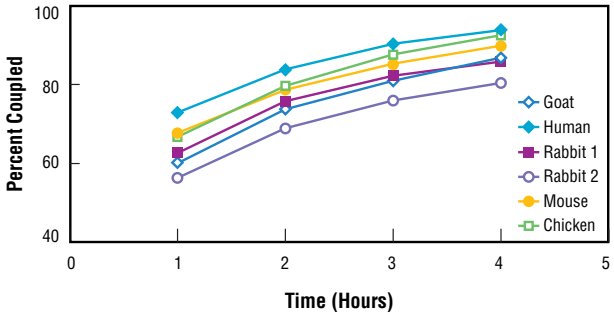
Seize® Primary Immunoprecipitation Kits

No species or subclass requirements and no antibody band interference.

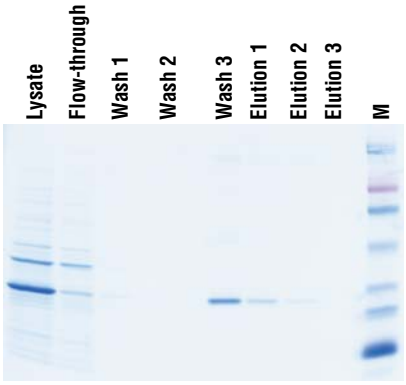
Seize® Primary Immunoprecipitation Kits eliminate the need to determine if an antibody species or subclass binds well to Protein A or Protein G. Using this kit, directly couple a primary antibody to the AminoLink® Plus Activated Support and create a reusable, immunoprecipitation (IP) resin that prevents the antibody from co-eluting with the target protein.

Highlights:

- Eliminates resin loss, providing more consistent results
- Covalent attachment of primary antibody prevents co-elution with the target protein
- Eliminates interference from antibody heavy- and light-chain bands on SDS-PAGE or Western blots
- Recover more target protein than obtained with the Seize® X Kit
- Couple any species and Ig subclass (chicken IgY, human IgE, mouse IgG_{2a}, etc.)
- Reuse expensive primary antibodies for up to 10 IPs
- Does not use a cross-linker to create an IP gel
- Complete kit formats – with or without M-PER® Mammalian Protein Extraction Reagent
- Use with purified antibodies, free from carrier protein



Antibody coupling efficiency of mammalian and avian antibody. Purified antibody (200 µg) from various species was coupled to 200 µl of AminoLink® Plus Coupling Gel (settled gel) at 1-hour intervals for 4 hours at room temperature. For the chicken antibody, 500 µg was used.



The Seize® Primary Kit produces exceptionally clean IP results.

Both the Seize® Primary and Seize® X IP Kits offer a reusable antibody-coupled resin resulting in no antibody heavy and light chain contamination. However, in the Seize® X IP Procedure, DSS is used to cross-link the antibody to the Protein A or Protein G resin. Because cross-linking often reduces the antibody-binding capacity, this step has been eliminated in the Seize® Primary IP Kit to allow for greater recovery of the target protein. While the Seize® Classic Kit method potentially yields a greater quantity of target protein, the presence of antibody heavy and light chains in the eluent can distort or hide the recovered target protein band on a polyacrylamide gel.

References

Kwon, Y.H., *et al.* (2002). *J. Biol. Chem.* **277**, 41417-41422.

Eberhardt, W., *et al.* (2002). *Mol. Endocrinol.* **16**, 1752-1766.

Ordering Information

Product #	Description	Pkg. Size
45335	**Seize® Primary Immunoprecipitation Kit** *Sufficient reagents to immobilize 10 different antibodies and to perform 20 IP reactions.** Includes: AminoLink® Plus Coupling Gel	Kit 2 ml
	Coupling Buffer	500 ml
	Quenching Buffer	60 ml
	Wash Buffer	60 ml
	Reducing Agent	0.5 ml
	Handee™ Microcentrifuge Tubes	150 ea.
	Handee™ Spin Cup Columns	12/pkg.
	Binding Buffer	500 ml
	Immunoprecipitation Sample Buffer	500 ml
	ImmunoPure® Elution Buffer	50 ml
	ImmunoPure® Lane Marker Sample Buffer, Non-reducing	5 ml
45332	**Seize® Primary Mammalian Immunoprecipitation Kit** *Sufficient reagents to lyse mammalian cells, immobilize 10 different antibodies and perform 20 IP reactions.** Kit includes same components as Product # 45335, but it also includes a 25 ml bottle of M-PER® Mammalian Protein Extraction Reagent	Kit
69700	**Handee™ Spin Cup Columns**	50 units
69715	**Handee™ Microcentrifuge Tubes, 1.5 ml**	72 tubes

**Based on a scale of 100-200 µl of settled gel per IP reaction. Kit can provide up to 200 IP reactions if more Handee™ Spin Cup Columns are purchased separately.*

Seize® Coated Plate Immunoprecipitation Kits

Pre-coated 96-well plates are easier to use and faster than traditional microtube methods.

Each kit includes 12 x 8-well strip plates

- More efficient processing of multiple samples and controls using multichannel pipettes
- Faster and easier washing of wells compared to single-tube matrix protocols
- Each strip allows for duplicates of a controlled series of samples or two separate controlled reactions

Pre-blocked plates

- Reduces nonspecific binding

More consistent results

- Uniform coating of protein makes binding of antibody and target more consistent

Immunoprecipitation (IP) experiments performed using these microplates can be performed more quickly relative to traditional tube-based methods. The more samples processed at one time, the more time saved. When processing just a few samples, this method is about 25% faster than traditional tube-based protocols. The processing time for 12 or more samples can be cut in half. Most of the time saved is in the washing and elution steps.

The 96-well strip plate is a convenient platform upon which to conduct a series of IP or co-immunoprecipitation (co-IP) experiments. IP reactions can involve as few as one to two samples or as many as 24 samples immunoprecipitated simultaneously on a single 96-well strip plate. Up to 24 samples can be performed on a single plate. Unused strips can be stored for future reactions.

The coated microplate approach to IP and co-IP:

- Offers excellent consistency and reproducibility of results
- Requires minimal optimizing of antigen and antibody dilutions due to antibody binding strategy
- Uses antibody and sample volumes comparable to tube-based protocols
- Easily recovers immunoprecipitated target samples for downstream analysis
- Includes easy-to-follow, robust protocol for first-time users

Each Seize® Coated Plate IP Kit listed includes:

- BupH™ Phosphate Buffered Saline, 2 packs
- Surfact-Amps® X-100 (10% Triton® X-100), 6 x 10 ml
- Elution Buffer, 50 ml
- Neutralization Buffer, 7 ml
- Uncoated 96-well Strip Plates (white), 2 each (for sample collection and neutralization)
- Plate Sealers, 18 sheets

Reference

Desai, S. and Hermanson, G. (1997). *Previews* **1(3)**, 2-7.

Ordering Information

Product #	Description	Pkg. Size
45350	**Seize® Protein A/G Coated Plate IP Kit** *Antibody binding capacity/well: 2.5 µg/100 µl coating volume. Sufficient capacity for downstream analysis of IP or co-IP proteins in-gel or by Western blot.*	Kit
	Includes: Protein A/G Coated Strip Plates	2 x 96-well
45355	**Seize® Protein G Coated Plate IP Kit** *Antibody binding capacity/well: 2.5 µg/100 µl coating volume. Sufficient capacity for downstream analysis of IP or co-IP proteins in-gel or by Western blot.*	Kit
	Includes: Protein G Coated Strip Plates	2 x 96-well
45360	**Seize® Streptavidin Coated Plate IP Kit** *Antibody binding capacity/well: 5 µg/100 µl coating volume. Sufficient capacity for downstream analysis of IP or co-IP proteins in-gel or by Western blot.*	Kit
	Includes: High Binding Capacity Streptavidin Coated Plates	2 x 96-well
	Biotin Blocking Buffer	2 x 15 ml

ProFound™ Co-Immunoprecipitation Kits

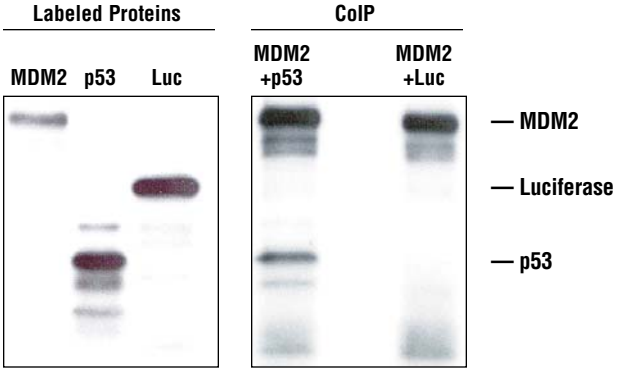
All the components needed to perform a properly controlled co-IP experiment.

Co-immunoprecipitation, or co-IP as it is widely known, is the gateway method to all other *in vitro* methods for protein interaction analysis. As a result, co-IP is among the most common and fastest growing *in vitro* approaches to protein:protein interaction discovery and verification in use today. For example, protein interactions discovered through the use of yeast two-hybrid experiments are most often confirmed by co-IP experiments. The ProFound™ Co-Immunoprecipitation Kit was configured to provide the essential tools to effectively perform a co-IP using the primary antibody of your choice. These kits provide those attempting a co-IP for the first time, as well as those experienced in the method, an ideal system for designing, constructing and performing a controlled co-IP experiment.

Benefits of target antibody immobilization

The heart of the new ProFound™ Co-Immunoprecipitation Kit is our AminoLink® Plus Coupling Gel that brings several benefits to the co-IP application.

- The AminoLink® Coupling Gel allows immobilization of a purified antibody against the target protein directly onto the matrix while retaining antibody activity.
- The antibody-coupled support retains the antibody during elution of the co-IP complex, an ideal feature for co-IP.
- Detection of the co-precipitated products on the gel is accomplished without interference from antibody fragments commonly encountered with other methods based on immobilized Protein A or Protein G supports.
- Covalent attachment of antibody conserves costly antibody and allows the matrix to be used repeatedly.



Co-IP of p53 and MDM2 with mouse MAb to MDM2 using components of the ProFound™ Co-Immunoprecipitation Kit. Purified mouse anti-MDM2 was coupled to AminoLink® Plus Coupling Gel. Luciferase, MDM2 and p53 were translated and ^{35}S labeled. *In vitro* translated p53 (5 µl) and MDM2 (5 µl) were combined and incubated at 30°C for 30 minutes. Co-IP was performed at 4°C for 2 hours with 60 µl anti-MDM2 antibody-coupled resin. Luciferase was used as a negative control protein to incubate with MDM2. Eluted proteins were resolved using a 4-20% SDS-PAGE gel and visualized by autoradiography.

Highlights:

- AminoLink® Plus Coupling Gel for covalent attachment of primary antibody prevents co-elution of antibody or antibody fragments with the target protein interactors
- Couple any purified antibody regardless of species or Ig subclass (chicken IgY, human IgE, mouse IgG_1, IgM, etc.)
- Physiologic co-IP buffer for optimal binding
- Control gel included – allows for a properly controlled co-IP experiment
- Spin cup columns for efficient washing and elution of small gel volumes
- Coupled antibody support reusable up to 10 times – saves precious antibody

Ordering Information

Product #	Description	Pkg. Size
23600	**ProFound™ Co-Immunoprecipitation Kit** *Sufficient material to immobilize up to 10 antibodies and perform a minimum of 40 co-IP reactions if 25 µl of antibody immobilized support is used. More co-IPs can be performed if smaller volumes are used.*	Kit
	Includes: AminoLink® Plus Coupling Gel, (4 ml of a 50% slurry)	2 ml settled gel
	Control Gel, (4 ml of a 50% slurry)	2 ml settled gel
	BupH™ Modified Dulbecco's PBS, (Coupling Buffer and Co-IP Buffer) (1 liter total volume)	2 packs
	Quenching Buffer	60 ml
	Wash Solution	60 ml
	Sodium Cyanoborohydride Solution (5 M)	0.5 ml
	Handee™ Spin-Cup Columns	50 ea.
	Handee™ Microcentrifuge Tubes, 1.5 ml	144 ea.
	IgG Elution Buffer	50 ml
	ImmunoPure® Lane Marker Sample Buffer, Non-Reducing (5X)	5 ml
23605	**ProFound™ Mammalian Co-Immunoprecipitation Kit** *Sufficient material to immobilize up to 10 antibodies and perform a minimum of 40 co-IP reactions if 25 µl of antibody immobilized support is used. More co-IPs can be performed if smaller volumes are used.* Includes same components as Product # 23600 and also includes:	Kit
	M-PER® Lysis Buffer	25 ml

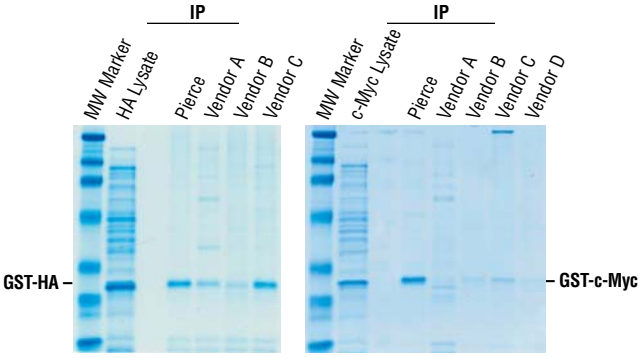
ProFound™ HA and c-Myc IP/Co-IP Kits

Confirm two-hybrid interactions.

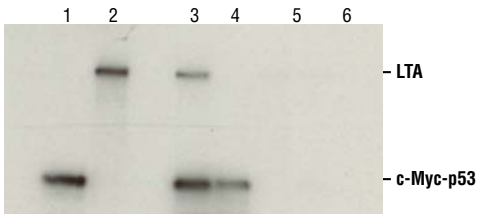
The new ProFound™ HA and c-Myc Tagged Protein Co-IP Kits include high-affinity, high-specificity antibodies coupled to agarose to enable capture of HA- or c-Myc-tagged proteins along with their binding partners. The covalent linkage between antibody and the resin produces results free from antibody contamination and with minimal background. The mammalian kits also include M-PER® Mammalian Protein Extraction Reagent, which quickly and gently lyses mammalian cells for easy extract preparation that is compatible with the co-immuno-precipitation (co-IP).

Highlights:

- Specific, high-affinity antibody provides high yield and minimal background
- No antibody contamination in eluted sample
- Control agarose gel included to test nonspecific binding or pre-clear sample
- Control tagged protein included to verify kit performance
- Complete kits for IP or co-IP, with no reagent formulation necessary
- Scalable for different antibody-gel and lysate requirements
- Includes Mini-Spin Columns for efficient washing and elution steps



Comparison of the effectiveness of anti-HA- and anti-c-Myc-coupled resins.
Anti-HA and anti-c-Myc resins from Pierce show high affinity and high specificity.



Co-IP of SV40 large T-antigen (LTA) with c-Myc-tagged p53.

Ordering Information

Product #	Description	Pkg. Size
23610	**ProFound™ HA-Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with an HA tag. The kit is supplied complete with an HA-tagged positive control lysate containing over-expressed GST-HA.* Includes: Product # 23612, ProFound™ HA-Tag IP/Co-IP Application Set, Product # 23613, HA-Tagged Positive Control, 500 µl	Kit
23615	**ProFound™ Mammalian HA-Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with an HA tag. The kit is supplied complete with a mammalian cell lysis buffer and an HA-tagged positive control lysate containing over-expressed GST-HA.* Includes: Product # 23617, ProFound™ Mammalian HA-Tag IP/Co-IP Application Set Product # 23613, HA-Tagged Positive Control, 500 µl	Kit
23620	**ProFound™ c-Myc-Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with a c-Myc peptide tag. Kit is supplied complete with a c-Myc-tagged positive control lysate containing over-expressed GST-c-Myc.* Includes: Product # 23622, ProFound™ c-Myc Tag IP/Co-IP Application Set Product # 23633, c-Myc Tagged Positive Control, 500 µl	Kit
23625	**ProFound™ Mammalian c-Myc Tag IP/Co-IP Kit** *Sufficient material to conduct 25 IP/co-IP assays using proteins expressed with a c-Myc peptide tag. Kit is supplied complete with a mammalian cell lysis buffer and a c-Myc-tagged positive control lysate containing over-expressed GST-c-Myc.* Includes: Product # 23627, ProFound™ Mammalian c-Myc Tag IP/Co-IP Application Set Product # 23633, c-Myc Tagged Positive Control, 500 µl	Kit

ImmunoPure® Classic IgG Orientation Kits

Available with either Immobilized Protein A or Immobilized Protein G.

Highlights:

- Allow the positioning of IgG with the antigen-binding sites directed outward, capturing passing antigen in the mobile phase
- Antibody's position is secured using the cross-linker DMP for fast and simple antigen purification
- Two kits allow you to select the protein (A or G) that binds best to your IgG subclass

Ordering Information

Product #	Description	Pkg. Size
44898	ImmunoPure® Protein A IgG Orientation Kit *Sufficient reagents for preparing 2 x 2 ml columns.* Maximum recommended loading capacity: 10 mg Rabbit IgG per column.	Kit
44896	ImmunoPure® Protein G IgG Orientation Kit *Sufficient reagents for preparing 2 x 2 ml columns.* Maximum recommended loading capacity: 10 mg Rabbit IgG per column.	Kit

ImmunoPure® Plus IgG Orientation Kits

Greater antibody-binding capacities than the Classic IgG Orientation Kits; available with either recombinant Protein A or Protein G.

Highlights:

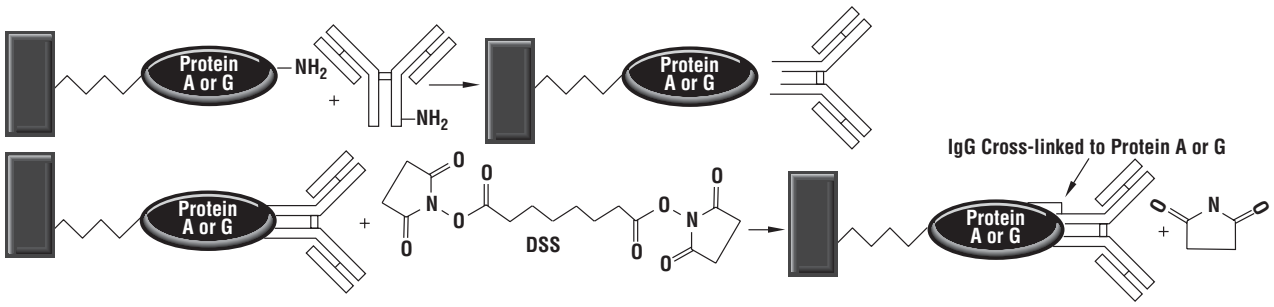
- DSS cross-linking system allows for higher antibody loading and less antibody leaching
- Allows the positioning of IgG with the antigen-binding sites directed outward, capturing passing antigen in the mobile phase
- Complete kits include all the reagents needed to immobilize the antibody

Reference

Venturi, M., *et al.* (2000). *J. Biol. Chem.* **275(7)**, 4734-4742.

Ordering Information

Product #	Description	Pkg. Size
44893	ImmunoPure® Recombinant Protein A IgG Plus Orientation Kit *Sufficient reagents for preparing 2 x 2 ml columns.* Maximum recommended loading capacity: 16 mg Rabbit IgG per column.	Kit
44990	ImmunoPure® Protein G IgG Plus Orientation Kit *Sufficient reagents for preparing 2 x 2 ml columns.* Maximum recommended loading capacity: 16 mg Rabbit IgG per column.	Kit



Affinity Procedures for **Contaminant Removal**

Following the primary purification procedure to obtain a sample of interest, secondary purification steps to remove contaminants may be required. The contaminants can be inhibitors, interfering substances or inappropriate buffers. A number of available affinity supports allow researchers to either specifically purify a protein of interest away from a complex mixture of biological molecules (positive selection) or remove specific contaminants from a sample containing a protein of interest (negative selection). Nearly any given affinity purification system can be used for either positive or negative selection, depending on whether the non-bound or eluted fraction is recovered. For example, immobilized Protein A can be used for general affinity purification of antibodies (positive selection), but it can also be used to selectively remove immunoglobulins from a sample in which they are considered a contaminant (negative selection). Following is a brief description of affinity-based systems for removal of contaminants by negative selection.

Removal of Detergent

However necessary the use of detergent may have been for initial cell lysis or membrane protein extractions, subsequent applications or experiments with the extracted proteins may require removal of some or all of the detergent. For example, although many water-soluble proteins are functional in detergent-solubilized form, membrane proteins are often modified and inactivated by detergent solubilization as a result of native lipid interactions having been disrupted. In some such cases, membrane protein function is restored when they are reconstituted into bilayer membranes by replacement of detergent with phospholipids or other membrane-like lipid mixtures.

The function of an individual protein can be studied in isolation if it is first purified and then reconstituted into an artificial membrane (although recovery of native orientation in the membrane is a major challenge). Even when restoration of protein function is not an issue, detergent concentration may have to be decreased in a sample to make it compatible with protein assays or gel electrophoresis.

Detergent removal can be attempted in a number of ways. Dialysis is effective for removal of detergents that have high CMCs and/or small aggregation numbers, such the *N*-octyl glucosides. Detergents with low CMCs and large aggregation numbers cannot be dialyzed since most of the detergent molecules will be in micelles that are too large to diffuse through the pores of the dialysis membrane; only excess monomer can be dialyzed. Ion exchange chromatography using appropriate conditions to selectively bind and elute the proteins of interest is another effective way to remove detergent. Sucrose density gradient separation also can be used.

Extracti-Gel™ D Detergent Removal Gel from Pierce allows fairly selective affinity-based removal of many different detergents from solutions.

Extracti-Gel® D Detergent Removing Gel

Makes detergent removal efficient, fast and easy with high protein recoveries, too.

Highlights:

- Allows relatively small detergent molecules to enter the gel matrix where they interact with a specially developed ligand capable of removing them from solution
- Low exclusion limit of the support overcomes nonspecific binding
- Recommended for use with biological macromolecules greater than 10 kDa
- Detergent is extracted without fear of losing a valuable protein
- Reusable affinity matrix can be regenerated up to three times
- Compatible with a wide variety of buffers, pH values 3.5-10
- Recovery of dilute protein solutions enhanced by use of a carrier protein

Applications:

- Reconstitution of proteoliposomes to study integral membrane proteins[1,2]
- Preparation of samples for mass spectroscopy[3,4]

Table 15. Detergent Binding Data

Detergent	Product #	Capacity (mg/ml gel)	Binding Conditions
Brij® -35	28316	80	100 mM Phosphate Buffer, pH 7.0
CHAPS	28300	50	0.05 M Tris Buffer, pH 9.0
SDS	28312	80	0.05 M Tris Buffer, pH 9.0
Triton® X-100	28314	57	100 mM Phosphate Buffer, pH 7.0
Tween® -20	28320	74	100 mM Phosphate Buffer, pH 7.0

References

1. Bubien, J.K., *et al.* (2001). *J. Biol. Chem.* **276**, 8557-8566.
2. Carman, C.V., *et al.* (2000). *J. Biol. Chem.* **275**, 10443-10452.
3. Rouse J.C. and Vath J.E. (1996). *Anal Biochem.* **238**, 82-92.
4. Gentile, F., *et al.* (1997). *J. Biol. Chem.* **272**, 639-646.

Ordering Information

Product #	Description	Pkg. Size
20208	**Extracti-Gel® D Detergent Removing Gel**	10 ml
20303	**Extracti-Gel® D Detergent Removing Gel**	100 ml
20346	**Extracti-Gel® D Detergent Removing Gel**	5 x 1 ml pre-packed columns

Removal of Albumin

Human serum albumin (HSA) can account for >60% of the total protein in serum samples and can have a concentration of ~40 mg/ml. This high concentration of albumin frequently interferes with analysis of proteins of biological interest. The SwellGel® Blue Albumin Removal Kit takes advantage of the albumin-binding properties of immobilized Cibacron® Blue F3GA Dye and is designed for rapid treatment of small sample volumes commonly used in proteomic studies. Each SwellGel® Blue Disc has the capacity (≥2 mg HSA) to remove the majority of albumin from 10-150 µl of serum in less than 15 minutes. The kit procedure has been optimized for removal of human serum albumin but will also effectively remove swine and sheep serum albumin. With a slight modification of the binding buffer, the SwellGel® Blue Kit can also be used to remove excess bovine, calf, goat and rat albumin. The product, however, is not recommended for mouse albumin.

SwellGel® Blue Albumin Removal Kit

Albumin-free serum samples in less than 15 minutes.

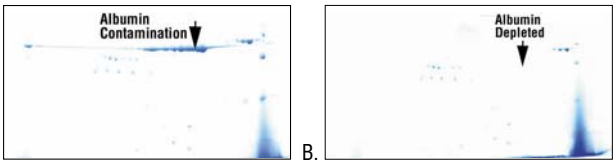
Traditionally, researchers have produced albumin-free samples using chromatographic methods involving multiple purification steps. In addition to involving lengthy and tedious procedures, these purification steps also tend to give low protein yields. To improve this process, Pierce scientists have developed a kit that takes advantage of both Cibacron® Blue Dye methods (widely used for removing HSA) and Pierce SwellGel® Technology. The SwellGel® Blue Albumin Removal Kit allows the researcher to remove albumin from human serum quickly and consistently while achieving higher protein yields. The new kit format also allows for greater ease in handling multiple samples.

With the addition of water, SwellGel® Blue Discs hydrate in less than 20 seconds to form a pre-equilibrated gel slurry that is equal to 200 µl of resin with a binding capacity for HSA of greater than 2 mg. The entire albumin removal procedure requires less than 15 minutes (Figure, page 58) using the Pierce SwellGel® Blue Albumin Removal Kit.

SwellGel® Blue Albumin Removal Kit Buffer is compatible with most downstream applications and does not interfere with protein assays or introduce contaminants for SDS-PAGE. The researcher adds the albumin-depleted serum to the appropriate sample buffer and the sample is ready for any application including 2-D gel applications.

SwellGel® Blue Albumin Removal Kit (continued)

Albumin-free serum samples in less than 15 minutes.

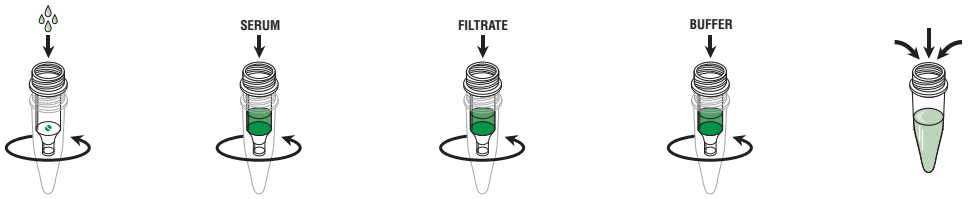


A. B.

SwellGel® Blue Albumin Removal Kit for 2-D gel analysis. Serum sample obtained by diluting 10 µl human serum with 40 µl TBS (Product # 28376) and loading 5 µl onto Gel A. Albumin-free sample obtained by diluting 50 µl human serum 1:1 with buffer, adding to a SwellGel® Blue Disc, washing the resin three times with 50 µl buffer, combining the fractions, and loading 5 µl onto Gel B. Both samples were focused using pH 4-7 isoelectric focusing (IEF) strips and run on 8-16% Tris-glycine gels.

Ordering Information

Product #	Description	Pkg. Size
89845	**SwellGel Blue Albumin Removal Kit***	Kit
	Sufficient reagents for 12-25 reactions.	
	Includes: SwellGel® Blue Albumin Removal Discs	25 Discs
	Binding/Wash Buffer	6.25 ml
	Handee™ Mini-Spin Columns	25 Columns
89846	**SwellGel® Blue Albumin Removal Kit***	Kit
	Sufficient reagents for 50-100 reactions.	
	Includes: SwellGel® Blue Albumin Removal Discs	100 Discs
	Binding/Wash Buffer	25 ml
	Handee™ Mini-Spin Columns	50 Columns



Step 1. Hydrate SwellGel® Disc in water. Spin.

Step 2. Add serum sample to the resin. Incubate 2 minutes.

Step 3. Recover filtrate and add to the resin. Incubate 2 minutes and spin.

Step 4. Add Binding/Elution Buffer. Spin. Wash 1-4 times.

Step 5. Combine desired fractions. Concentrate if needed.

Total Time: 10-15 minutes

Removal of Endotoxin

Endotoxins are pyrogenic lipopolysaccharide (LPS) components of gram-negative bacteria. Because these bacteria are ubiquitous, it is not surprising that endotoxins are frequent contaminants of biochemical preparations. Maintaining a low endotoxin concentration is extremely important for *in vivo* studies. Even at concentrations of nanograms per milliliter, they have been shown to exert potent biological effects in research animals and cultured cells. Endotoxin contamination usually is measured as endotoxin units (EU), where 1 EU corresponds to a concentration of endotoxin (usually about 0.1 ng/kg body weight) sufficient to generate a pyrogenic reaction.

Eliminating endotoxin contamination in aqueous and physiological solutions is a difficult and often expensive process. Ultrafiltration techniques work well to remove most pyrogens from buffer solutions. However, removal of endotoxin from solutions containing proteins or other macromolecules of interest is much more difficult. Complete removal of all contaminating pyrogens is not feasible; the best that can be expected is a reduction of endotoxins to tolerable levels for a given study system. Typically, a pyrogen-poor (i.e., safe for use) preparation of protein will contain less than 1.0 EU per milligram of protein.

Detoxi-Gel™ Endotoxin Removing Gel uses immobilized polymixin B to bind and remove endotoxins from solutions. The product is commonly used to remove endotoxins from protein solutions, cell culture media, solutions containing pharmacological components and aqueous buffers. Using 1 ml of the Detoxi-Gel™ Support, < 5 EU should be present in the flow through following a 10,000 EU/ml challenge. This corresponds to an efficiency of greater than 99%.

Certain substances and proteins in solution bind strongly to endotoxin, thereby either decreasing binding capacity of Detoxi-Gel™ Support or resulting in loss of protein from the sample (i.e., the protein remains bound to endotoxin when the endotoxin binds to the immobilized polymixin B). This is especially true of proteins like bovine serum albumin (BSA) and ovalbumin. BSA is a common component of tissue media and, as such, it is also often a source of endotoxin contamination.

Detoxi-Gel™ Endotoxin Removing Gel

Eliminate worries about endotoxins interfering with your test results.

Highlights:

- Efficiency of endotoxin removal is 99.75% or greater in one pass with a 10,000 EU challenge on a 1 ml Detoxi-Gel™ Column
- High stability – no loss in binding capacity of a Detoxi-Gel™ Column after even 10 regenerations
- Can be regenerated by stripping off the endotoxins with a 1% deoxycholate solution in pyrogen-poor water
- Contains an immobilized ligand that binds and removes pyrogens from solutions
- Extremely versatile, reducing endotoxin levels in protein solutions, cell culture media, solutions containing pharmacological components and aqueous buffers

Ordering Information

Product #	Description	Pkg. Size
20344	Detoxi-Gel™ AffinityPak™ Prepacked Columns	5 x 1 ml
20339	Detoxi-Gel™ Endotoxin Removing Gel	10 ml
20340	Detoxi-Gel™ Endotoxin Removing Gel	1,000 ml

References

Chigaev, A., *et al.* (2003). *J. Biol. Chem.* **278**, 38174-38182.
Gao, B. and Tsan, M. (2003). *J. Biol. Chem.* **278**, 22523-22529.
Zhang, J., *et al.* (2000). *FASEB J.* **14**, 2589-2600.
Hahn-Dantona, E., *et al.* (2001). *J. Biol. Chem.* **276**, 15498-15503.

Removal of Nonspecific Antibodies

Because they contain mixtures of immunoglobulins, preparations of polyclonal antibodies from serum or other samples often have cross-reactivity to unintended targets in the sample being probed in Western blot or ELISA. It is important to purchase or prepare antibodies that do not cross-react in this way. For example, by passing a rabbit anti-mouse IgG polyclonal antibody sample over a column of immobilized human serum proteins, one can ensure that the resulting antibody will react only to mouse IgG primary antibody without cross-reacting with human immunoglobulins in the sample. Pierce offers selected antibodies that have been pre-adsorbed in this way to prevent cross-reactivity to unintended immunoglobulin species. Such pre-adsorption of antibodies is a form of negative selection affinity purification wherein the immobilized ligand is a mixture of proteins. For more information on pre-adsorbed antibodies, order literature # 1600923 from Pierce customer service.

Cross-reactivity of antibodies to bacterial proteins is a common problem for researchers investigating recombinant proteins prepared in *Escherichia coli*. The possibility of such cross-reactivity can be reduced or eliminated by removing immunoglobulins in the polyclonal antibody sample that bind to proteins from *E. coli.* Pierce offers the Immobilized *E. coli* Lysate Kit for this purpose. Proteins from total lysate of *E. coli* strain BMH 71-18 have been immobilized onto a 4% cross-linked agarose support.

Immobilized *E. coli* Lysate and Kit

For clean, easy removal of E. coli*-reactive antibodies.*

High background, or low signal:noise ratio, is often a problem when screening libraries. Crude antisera and ascites fluid often contain IgG components that bind to *Escherichia coli* proteins. This could be especially problematic if the titer or binding affinity of the *E. coli* binding antibodies is higher than that of the antibody to the protein of interest, resulting in false positives.

To make removal of *E. coli* antibodies cleaner and easier, Pierce has immobilized *E. coli* proteins on a solid support. When a sample is passed over the column, the purified antibody is collected in the void volume. The contaminating *E. coli* antibodies are absorbed onto the matrix, and the purified antisera is collected.

Ordering Information

Product #	Description	Pkg. Size
44938	Immobilized *E. coli* Lysate *Gel of* E. coli *proteins from strain BMH 71-18 immobilized on 4% cross-linked beaded agarose.*	5 ml
44940	Immobilized *E. coli* Lysate Kit	Kit
	Includes: Prepacked Column of *E. coli* Lysate	2 ml
	BupH™ Tris Buffered Saline	500 ml
	Regeneration Buffer Column Extender	250 ml

Other **Immobilized Ligands**

Immobilized Soybean Trypsin Inhibitor

For effective removal of trypsin, chymotrypsin and elastase from protein digests.

Applications:

- Purifying trypsin, chymotrypsin and elastase[1,2]
- Removing proteases from activated pancreatic juices[3]

References

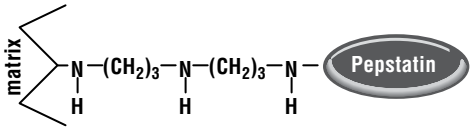
1. Feinstein, G., *et al.* (1974). *Euro. J. Biochem.* **43(3)**, 569-581.
2. Peterson, L.M., *et al.* (1976). *Biochemistry* **15(12)**, 2501-2508.
3. Reeck, G.R., *et al.* (1971). *Biochemistry* **10(25)**, 4690-4698.

Ordering Information

Product #	Description	Pkg. Size
20235	**Immobilized Soybean Trypsin Inhibitor** STI coupled to spherical 4% beaded agarose. Capacity: Minimum of 6 mg trypsin/ml of gel Supplied in glycerol with 0.05% sodium azide	2 ml gel

Immobilized Pepstatin

An excellent cathepsin binding matrix.



R = (4-amino-3-hydroxy-6-methyl) heptanoic acid

Immobilized Pepstatin

Reference

Helseth, Jr., D.L. and Veis, A. (1984). *Proc. Natl. Acad. Sci. USA* **81**, 3302-3306.

Ordering Information

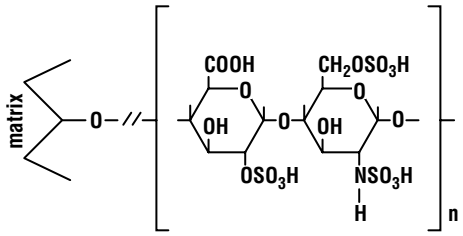
Product #	Description	Pkg. Size
20215	**Immobilized Pepstatin Gel** Support: Cross-linked 6% beaded agarose Spacer: Diaminodipropylamine Capacity: 1-2 mg of pepsin/ml of gel	5 ml

Immobilized Heparin on Agarose

Use to isolate many blood proteins that have enzymatic activities.

Applications:

- Enrich lysates for nucleic acid-binding proteins
- Isolate many blood proteins



Immobilized Heparin on Agarose

Reference

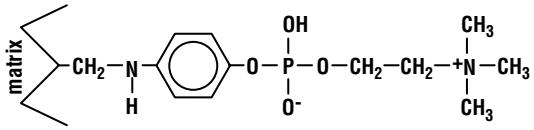
Smith, P.K., *et al.* (1980). *Anal. Biochem.* **109**, 466-473.

Ordering Information

Product #	Description	Pkg. Size
20207	**Immobilized Heparin Gel** Support: 4% beaded agarose Loading: ≥ 0.2 mg of heparin/ml of gel (determined by the colorimetric method)	10 ml

Immobilized *p*-Aminophenyl Phosphoryl Choline

For C-reactive protein binding.



Immobilized *p*-Aminophenyl Phosphoryl Choline

Reference

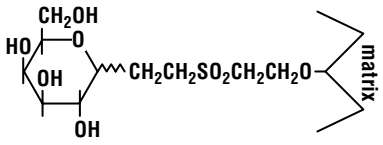
Robey, F.A. and Liu, T.Y. (1981). *J. Biol. Chem.* **256**, 969-975.

Ordering Information

Product #	Description	Pkg. Size
20307	**Immobilized *p*-Aminophenyl Phosphoryl Choline Gel** Support: Cross-linked 6% beaded agarose Capacity: ≥ 5-11 mg of human C-reactive protein/ml of gel	5 ml

Immobilized D-Galactose

For castor bean lectin binding.



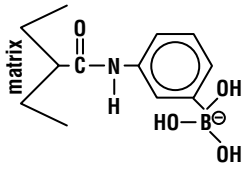
Immobilized D-Galactose

Ordering Information

Product #	Description	Pkg. Size
20372	**Immobilized D-Galactose Gel** Support: 6% beaded agarose Capacity: ≥ 8 mg castor bean lectin/ml of gel	5 ml

Immobilized Boronic Acid Gel

For ribonucleoside isolation.



Immobilized Boronic Acid

References

Vlassara, H., *et al.* (1981). *Proc. Natl. Acad. Sci. USA* **78**, 5190-5192.
Gehrke, C.W., *et al.* (1978). *J. Chromatogr.* **150**, 455-476.

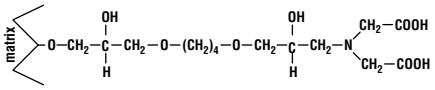
Ordering Information

Product #	Description	Pkg. Size
20244	**Immobilized Boronic Acid Gel** Support: Polyacrylamide spherical beads Spacer: *m*-aminophenyl group Loading: 100 µmoles boronate/ml of gel	10 ml

Additional Affinity Supports

Immobilized Iminodiacetic Acid

A highly effective ion chelating support.



Immobilized Iminodiacetic Acid

Reference
Shriner, C.L. and Brautigan, D.L. (1984). *J. Biol. Chem.* **259**, 11383-11390.

Ordering Information

Product #	Description	Pkg. Size
20277	**Immobilized Iminodiacetic Acid Gel** Support: 4% beaded agarose Spacer: 1,4-Butanediol diglycidyl ether	10 ml

MagnaBind™ Beads and Supports

A convenient method for isolating biomolecules using affinity binding, while retaining biological activity.

Highlights:

- MagnaBind™ Beads are available pre-coated with streptavidin or secondary antibodies, or derivatized with carboxyl or amine groups
- Beads do not aggregate because they respond well to weak magnetic fields but have no magnetic memory
- Most separations require a short five- to 10-minute bench-top procedure
- Surface area of bead is >100 m^2/g
- 1 x 10^8 particles/mg
- 5 mg beads/ml
- 1-4 µm diameter
- Simple sterilization

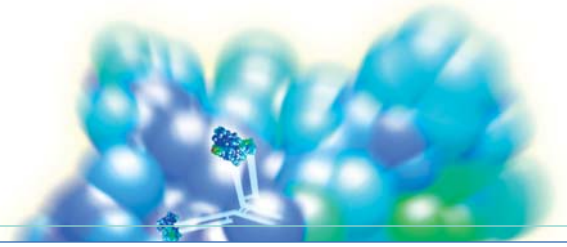
Applications:

- Cell sorting using positive or negative selection
- Protein purification or immunoassays using direct or indirect methods

Reference
Chaudhuri, T.K., *et al.* (2001). *Cell* **107**, 235-246.
Newey, S.E., *et al.* (2001). *J. Biol. Chem.* **276**, 6645-6655.
Xu, X., *et al.* (2001). *J. Biol. Chem.* **276**, 43221-43230.

Ordering Information

Product #	Description	Pkg. Size
21344	**MagnaBind™ Streptavidin Beads**	5 ml
21354	**MagnaBind™ Goat Anti-Mouse IgG Beads**	50 ml
21356	**MagnaBind™ Goat Anti-Rabbit IgG Beads**	50 ml
21353	**MagnaBind™ Carboxyl Derivatized Beads**	5 ml
21352	**MagnaBind™ Amine Derivatized Beads**	5 ml
21358	**MagnaBind™ Magnet for 96-Well Plate Separator**	1 magnet
21357	**MagnaBind™ Magnet for 1.5 ml Microcentrifuge Tube**	1 magnet
21359	**MagnaBind™ Magnet for 6 x 1.5 ml Microcentrifuge Tubes**	1 magnet



Polystyrene Hydrazide Beads

Ideal for coupling antibodies.

Highlights:

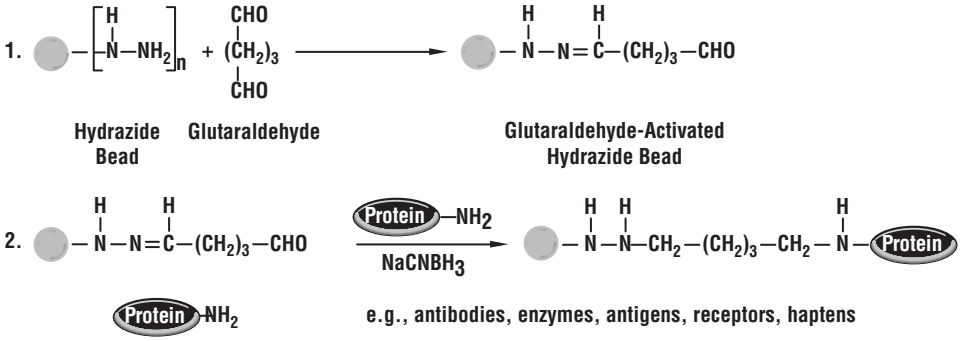
- This method couples antibodies via carbohydrate groups in the Fc region, resulting in site-directed immobilization in one step
- Coupling of IgG to Hydrazide Beads can also be done via glutaraldehyde activation and reduction of the Schiff base, upon introduction of an amine-containing species with $NaCNBH_3$

Reference

O'Shannessy, D.J. and Hofmann, W.L. (1987). *Biotech. Appl. Biochem.* **9**, 488-496.

Ordering Information

Product #	Description	Pkg. Size
20202	**Hydrazide Beads** Hydrazide derivatized uniform, nonporous spherical polystyrene beads Bead Diameter: 1/4" Loading: approximately 3 µmoles of hydrazide function per bead	250 beads



Polystyrene Beads, Underivatized

For high protein loading in a variety of immobilization applications including RIA and immunoassays.

Highlights:

- Proteins can be loaded onto the bead's surface by way of hydrophobic adsorption
- Special finish greatly increases the surface area of the bead, making high-protein loading possible
- A 1/4-inch bead is capable of adsorbing 1 µg of protein per bead from a 2 µg-per-bead solution of protein
- Hydrophobic interactions occur at alkaline pH values in the range of 8.5-10 within one to two hours at room temperature

References of General Interest

Hermanson, G.T., *et al.* (1992). *Immobilized Affinity Ligand Techniques.* California: Academic Press, Inc. (Product # 22230)

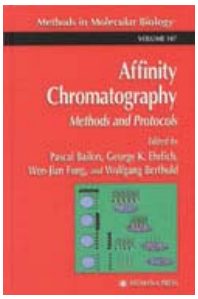
Dean, P.D.G., *et al.* (1985). *Affinity Chromatography: A Practical Approach.* Oxford: IRL Press.

Ordering Information

Product #	Description	Pkg. Size
23804	**Polystyrene Beads, Underivatized** Spherical, nonporous beads Bead Diameter: 1/4"	500 beads

Additional Affinity

Affinity Chromatography Methods and Protocols



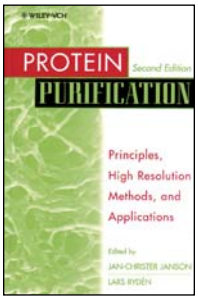
This text covers the most powerful affinity methods in use today. Techniques range from widely practiced immunoaffinity chromatography to state-of-the-art phage display applied to the discovery of affinity ligands and drugs. Each topic covered includes an explanation of principles, a materials list and step-by-step instructions.

Ordering Information

Product #	Description
20053	Affinity Chromatography Methods and Protocols Bailon, P., *et al.*, Eds., Published by Humana Press, 240 pages, Hardcover

Protein Purification: Principles, High-Resolution Methods, and Applications

Contemporary coverage of chromatographic and electrophoretic protein separation and characterization methods.



Joining principles, methods and applications, this updated edition of Protein Purification gives researchers ready access to a magnitude of important high-resolution biochemical separation techniques. Subjects include gel filtration and ion exchange chromatography, immunoelectrophoresis and capillary electrophoresis, and protein elution and blotting methods.

Ordering Information

Product #	Description
20032	Protein Purification: Principles, High-Resolution Methods, and Applications Janson, J.-C. and Rydén, L., Eds., Published by John Wiley & Sons, Inc., 1998, 695 pages, Hardcover

Immobilized Affinity Ligand Techniques

All the "recipes" for successful affinity matrix preparation.

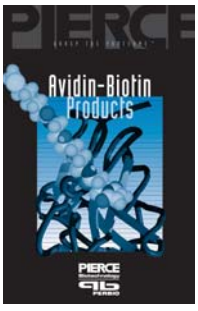
This book is a practical guide for reviewing the potential applications of affinity chromatography. Unlike other chemical guides, emphasis is placed on antibody fragmentation and immobilized antibody supports used for separation.

The following applications for affinity supports are discussed:
- Purification of complex solutions
- Scavenging reagents to remove unwanted contaminants
- Modification or catalysis to effect specific transformations
- Separation tools for analytical determinations

Ordering Information

Product #	Description
22230	Immobilized Affinity Ligand Techniques Hermanson, G.T., *et al.*, Published by Academic Press, Inc., 1992, 450 pages, Comb-bound

FREE Avidin-Biotin Product Guide



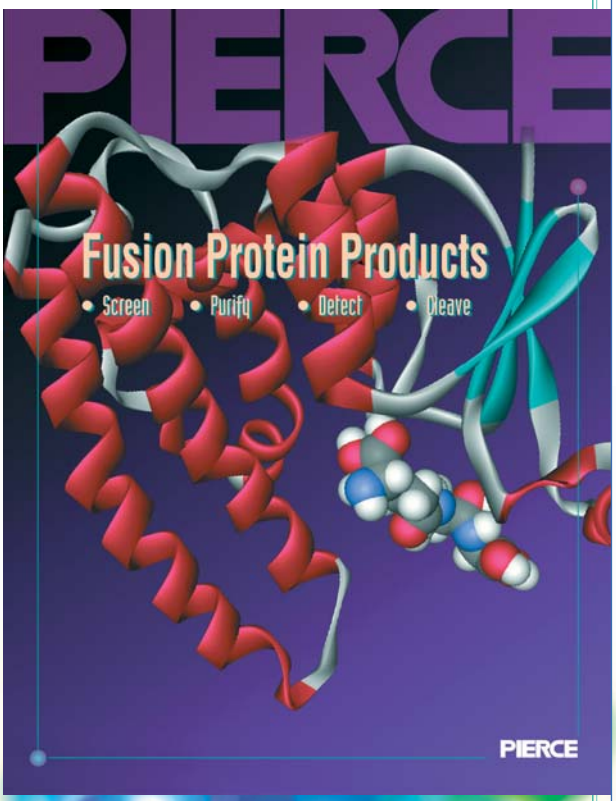
This reference guide brings together everything needed to biotinylate cell-surface proteins, purify a biotinylated target, detect a biotinylated antibody and perform many other applications. It includes dozens of references along with protocols, troubleshooting tips, selection guides and a complete listing of available tools. Because the Avidin-Biotin system can be used in so many ways, you'll want to keep this booklet close at hand!

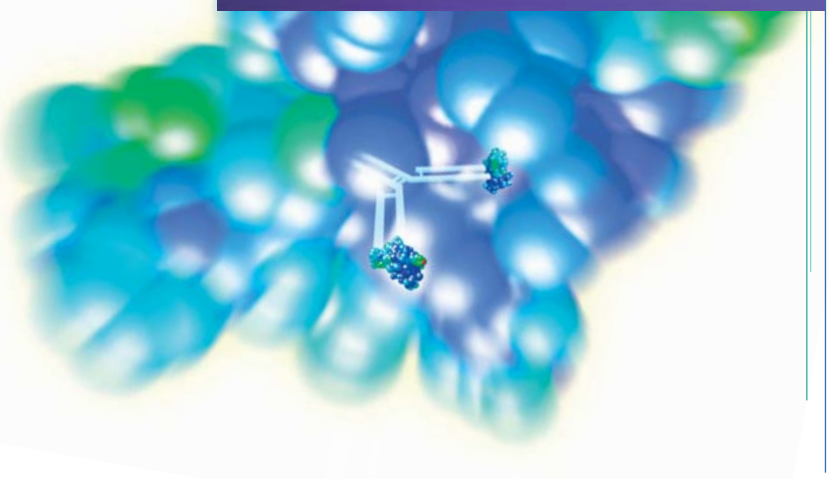
Ordering Information

Product #	Description
1600941	Avidin-Biotin Products Guide

Affinity Purification of **Fusion-Tagged Proteins**

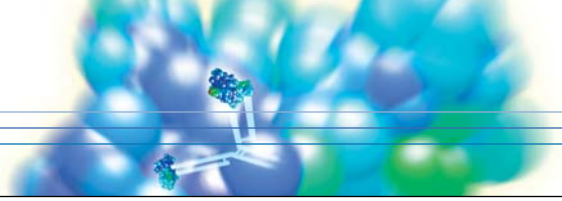
Pierce's new 12-page Fusion Protein Handbook (Product # 1600789) provides protocols and data for fusion protein purification, screening and detection and protein:protein interaction products and kits. Featured techniques include pull-down assays, GTPase activation, cell lysis and protein purification and orientation for polyhistidine-, GST-, HA-, c-Myc- and MBP-tagged targets. The handbook is an essential resource for any laboratory working with fusion proteins. To request a free copy of the handbook, log on to *www.piercenet.com* or call 800-874-3723 or 815-968-0747. Outside the United States, contact your local Perbio Science branch office or distributor.





Additional Affinity Supports

Product Type	Product Name	Product #	Pkg. Size	Support
Antibody Purification – Purify a wide variety of monoclonal and polyclonal antibodies from serum, culture supernatant or ascites fluid.				
Protein A	ImmunoPure® Immobilized Protein A	20333, 20334	5 ml resin, 25 ml resin	6% agarose
	AffinityPak™ Protein A Columns	20356	5 x 1 ml columns	6% agarose
	ImmunoPure® (A) IgG Purification Kit	44667	5 x 1 ml columns + reagents	6% agarose
	ImmunoPure® Immobilized Protein A	20338	5 ml resin	Trisacryl® GF-2000
	UltraLink® Immobilized Protein A[1]	53139	5 ml resin	Polyacrylamide
	ImmunoPure® Immobilized Protein A Plus	22811, 22812	5 ml resin, 25 ml resin	6% agarose
	AffinityPak™ Protein A Columns	22814	5 x 1 ml columns	6% agarose
	ImmunoPure® (A) Plus IgG Purification Kit	44679	5 x 1 ml columns + reagents	6% agarose
	UltraLink® Immobilized Protein A Plus[1]	53142	5 ml resin	Polyacrylamide
	Immobilized Recomb® Protein A	20365, 20366	5 ml resin, 25 ml resin	6% agarose
	NAb™ Protein A Spin Purification Kit	45200	1 ml resin + reagents	6% agarose
Protein G	ImmunoPure® Immobilized Protein G	20398, 20399	2 ml resin, 10 ml resin	6% agarose
	ImmunoPure® (G) IgG Purification Kit	44441	1 x 2 ml column + reagents	6% agarose
	UltraLink® Immobilized Protein G[1]	53125, 53126	2 ml resin, 10 ml resin	Polyacrylamide
	UltraLink® AffinityPak™ Immobilized Protein G[1]	53127	2 x 2 ml columns	Polyacrylamide
	ImmunoPure® Immobilized Protein G Plus	22851, 22852	2 ml resin, 10 ml resin	6% agarose
	UltraLink® Immobilized Protein G Plus[1]	53128	2 ml resin	Polyacrylamide
	NAb™ Protein G Spin Purification Kit	45201	1 ml resin + reagents	6% agarose
Protein A/G	ImmunoPure® Immobilized Protein A/G	20421, 20422	3 ml resin, 15 ml resin	6% agarose
	ImmunoPure® A/G IgG Purification Kit	44902	1 x 2 ml column + reagents	6% agarose
	UltraLink® Immobilized Protein A/G[1]	53132, 53133	2 ml resin, 10 ml resin	Polyacrylamide
	UltraLink® Immobilized Protein A/G Plus[1]	53135	2 ml resin	Polyacrylamide
Protein L	ImmunoPure® Immobilized Protein L	20510	2 ml resin	6% agarose
	AffinityPak™ Protein L Columns	20540	2 x 2 ml columns	6% agarose
	ImmunoPure® (L) IgG Purification Kit	20550	1 x 2 ml column + reagents	6% agarose
	ImmunoPure® Immobilized Protein L Plus	20520	2 ml resin	6% agarose
	NAb™ Protein L Spin Purification Kit	20530	1 ml resin + reagents	6% agarose
T-Gel™ Products	T-Gel™ Absorbent	20500	10 ml resin	6% agarose
	T-Gel™ Purification Kit	44916	4 x 3 ml column + reagents	6% agarose
Jacalin	Immobilized Jacalin	20395	5 ml resin	6% agarose
MBP	ImmunoPure® Immobilized MBP	22212	10 ml resin	4% agarose
	ImmunoPure® IgM Purification Kit	44897	1 x 5 ml column + reagents	4% agarose
	UltraLink® Immobilized MBP[1]	53123	5 ml resin	Polyacrylamide
Antibody Affinity Purification – Immobilize an antigen, then use it to purify specific antibodies. Increases the signal-to-noise ratio for cleaner assay results.				
Amine-reactive	AminoLink® Plus Immobilization Kit	44894	5 x 2 ml columns + reagents	4% agarose
	AminoLink® Plus Coupling Gel	20501	10 ml resin	4% agarose
	AminoLink® Kit	44890	5 x 2 ml columns + reagents	4% agarose
	AminoLink® Coupling Gel	20381, 20382	10 ml resin, 50 ml resin	4% agarose
	UltraLink® Immobilization Kit[1]	46500	5 x 2 ml columns + reagents	Polyacrylamide
	UltraLink® Biosupport Medium[1]	53112, 53110, 53111	2 ml resin, 10 ml resin, 50 ml resin	Polyacrylamide
Sulfhydryl-reactive	SulfoLink® Kit	44895	5 x 2 ml columns + reagents	6% agarose
	SulfoLink® Coupling Gel	20401, 20402	10 ml resin, 50 ml resin	6% agarose
	UltraLink® Iodoacetyl[1]	53155	10 ml resin	Polyacrylamide
Carbohydrate-reactive	CarboLink™ Kit	44900	5 x 2 ml columns + reagents	6% agarose
	CarboLink™ Coupling Gel	20391	10 ml resin	6% agarose
	UltraLink® Hydrazide[1]	53149	10 ml resin	Polyacrylamide
Carboxyl-reactive	CarboxyLink™ Coupling Kit	44899	5 x 2 ml columns + reagents	4% agarose
	CarboxyLink™ Coupling Gel	20266	25 ml resin	4% agarose
	UltraLink® EDC/DADPA Immobilization Kit[1]	53154	5 x 2 ml columns + reagents	Polyacrylamide
Active hydrogen-reactive	PharmaLink™ Immobilization Kit	44930	5 x 2 ml columns + reagents	6% agarose
GST Orientation	GST Orientation Kit	78201	2 x 2 ml columns + reagents	4% agarose
Contaminant Antibody Removal – Minimize background problems by removing cross-reactive antibodies.				
Anti-*E. coli* antibodies	Immobilized *E. coli* Lysate	44938	5 ml resin	4% agarose
	Immobilized *E. coli* Lysate Kit	44940	2 ml column + reagents	4% agarose
Anti-GST Antibodies	Immobilized GST	20205	2 x 2 ml column	6% agarose
	Immobilized GST	20211	5 ml resin	6% agarose
Immunoprecipitation/Pull-down – Cleanly and efficiently isolate interacting proteins using these innovative products.				
Immunoprecipitation Kits	Seize® Primary Immunoprecipitation Kit	45335	Reagents for 20+ IPs	6% agarose
	Seize® Primary Mammalian Immunoprecipitation Kit	45332	Reagents for 20+ IPs	6% agarose
	Seize® X Protein A Immunoprecipitation Kit	45215	Reagents for 40 IPs	6% agarose
	Seize® X Protein G Immunoprecipitation Kit	45210	Reagents for 40 IPs	6% agarose
	Seize® Classic Protein A Immunoprecipitation Kit	45213	Reagents for 50 IPs	6% agarose
	Seize® Classic Protein G Immunoprecipitation Kit	45218	Reagents for 50 IPs	6% agarose
	ProFound™ Co-Immunoprecipitation Kit	23600	Reagents for 40 IPs	6% agarose
	ProFound™ Mammalian Co-Immunoprecipitation Kit	23605	Reagents for 40 IPs	6% agarose
Pull-down Kits	ProFound™ Pull-Down PolyHis Protein:Protein Interaction Kit	21277	Reagents for 25 pull-downs	4% agarose
	ProFound™ Pull-Down GST Protein:Protein Interaction Kit	21516	Reagents for 25 pull-downs	4% agarose
	ProFound™ Pull-Down Biotinylated Protein:Protein Interaction Kit	21115	Reagents for 25 pull-downs	4% agarose
Magnetic Isolation	MagnaBind™ Goat Anti-Mouse IgG Beads	21354	50 ml	Iron oxide
	MagnaBind™ Goat Anti-Rabbit IgG Beads	21356	50 ml	Iron oxide
	MagnaBind™ Streptavidin Beads	21344	5 ml	Iron oxide



Approximate Binding Capacity	Applications / Features
12-19 mg human IgG/ml resin	Purify monoclonal and polyclonal IgG from serum, culture supernatant or ascites fluid
12-19 mg human IgG/ml resin	Ideal support to purify rabbit IgG
12-19 mg human IgG/ml resin	
>15 mg human IgG/ml resin	
>16 mg human IgG/ml resin	
>35 mg human IgG/ml resin	
>35 mg human IgG/ml resin	
>35 mg human IgG/ml resin	
>30 mg human IgG/ml resin	
>12 mg human IgG/ml resin	
~1 mg IgG/purification	
11-15 mg human IgG/ml resin	Purify monoclonal and polyclonal IgG from serum, culture supernatant or ascites fluid
11-15 mg human IgG/ml resin	Broader species specificity than Protein A with strong binding to Mouse IgG1 and human IgG3
>20 mg human IgG/ml resin	Binds only IgG isotype antibodies
>20 mg human IgG/ml resin	
>20 mg human IgG/ml resin	
>25 mg human IgG/ml resin	
~1 mg IgG/purification	
>7 mg human IgG/ml resin	Purify monoclonal and polyclonal IgG from serum, culture supernatant or ascites fluid
>7 mg human IgG/ml resin	Broadest specificity-combines the binding specificity of Protein A and Protein G
>20 mg human IgG/ml resin	
>28 mg human IgG/ml resin	
4-5 mg human IgG/ml resin	Purify monoclonal and polyclonal antibodies of all classes from serum, culture supernatant or ascites fluid
4-5 mg human IgG/ml resin	Purify single-chain variable fragments (ScFv) or Fab fragments
4-5 mg human IgG/ml resin	Binds only to antibodies with specific kappa light chains (mouse k1, human k1, k3, k4)
8-10 mg human IgG/ml resin	
~1 mg IgG/purification	
~20 mg Ig/ml resin	Purify antibodies from serum, culture supernatants or ascites fluid of a wide variety of species
~20 mg Ig/ml resin	Gentle elution conditions preserve antibody activity
1-3 mg human IgA/ml resin	Purify human IgA1 without contaminating IgG or IgM
~1 mg IgM/ml resin	Purify IgM in a single step
~1 mg IgM/ml resin	
>0.75 mg IgM/ml resin	
Range of 1-25 mg protein/ml resin	Immobilize any antigen on a rigid support with high flow rates through exposed lysine residues for antibody purification
0.1-2 mg peptide/ml resin	Stable, uncharged linkage for maximum binding specificity
Range of 1-20 mg protein/ml resin	Immobilize any antigen through exposed lysine residues for antibody purification
0.1-2 mg peptide/ml resin	Stable, uncharged linkage for maximum binding specificity
Range of 1-30 mg protein/ml resin	Immobilize any antigen through exposed lysine residues for antibody purification
0.1-2 mg peptide/ml resin	Rigid support for medium pressure applications
Range of 1-10 mg protein/ml resin	Immobilize peptide antigens with a terminal cysteine residue for antibody purification
0.1-2 mg peptide/ml resin	Long spacer arm reduces steric hindrance for maximal antibody binding
	Immobilize proteins that contain free cysteine residues
Range of 1-5 mg glycoprotein/ml resin	Immobilize glycoproteins or carbohydrates on a support for antibody purification
	Antigens are attached specifically through sugar groups
Range of 1-10 mg protein/ml resin	Immobilize antigens through aspartic and glutamic acid residues or the carboxy terminus
0.1-2 mg peptide/ml resin	Long spacer arm reduces steric hindrance for maximal antibody binding
Variable, up to 20 µmol/ml gel	Immobilize drugs and other organic molecules with no available reactive groups for antibody purification
1-10 mg fusion protein/column	Purify and covalently immobilize GST fusion proteins with a single column for antibody purification
~1 mg *E. coli* lysate/ml resin	Remove *E. coli*-reactive antibodies to reduce background when screening
~1 mg *E. coli* lysate/ml resin	
~0.5 mg anti-GST/ml resin	Prepare antibodies specific to the fusion protein rather than to the fusion tag by removing the GST-reactive antibodies
~0.5 mg anti-GST/ml resin	
25-400 µg antibody/IP	Isolate proteins using any antibody and without antibody bands interfering with protein analysis on the gel
25-400 µg antibody/IP	Enhances co-immunoprecipitation experiments by removing antibody bands from the analysis
50-500 µg antibody/IP	Isolate proteins using antibodies that bind to Protein A or G without antibody bands interfering with protein analysis on the gel
50-500 µg antibody/IP	Enhances co-immunoprecipitation experiments by removing antibody bands from the analysis
50-500 µg antibody/IP	Convenient format increases washing efficiency and requires less time than traditional immunoprecipitation
50-500 µg antibody/IP	
25-400 µg antibody/IP	Enhances co-immunoprecipitation experiments by removing antibody bands from the analysis
25-400 µg antibody/IP	Control procedures ensure that interactions are real
> 10 mg fusion protein/ml resin	Isolate interacting proteins more efficiently with a tagged bait protein
> 8 mg fusion protein/ml resin	Saves time compared to traditional pull-down experiments
> 5 mg biotinylated BSA/ml resin	
~0.2 mg mouse IgG/ml resin	Save time by performing immunoprecipitation or pull-down experiments magnetically
~0.2 mg rabbit IgG/ml resin	
~2 µg biotin/ml resin	

Additional Affinity Supports

Product Type	Product Name	Product #	Pkg. Size	Support
Activated Affinity Supports – Immobilize virtually any molecule on a solid support. Then use it in a batch or column method to purify its binding partners.				
Amine-reactive	AminoLink® Plus Immobilization Kit	44894	5 x 2 ml columns + reagents	4% agarose
	AminoLink® Plus Coupling Gel	20501	10 ml resin	4% agarose
	AminoLink® Kit	44890	5 x 2 ml columns + reagents	4% agarose
	AminoLink® Coupling Gel	20381, 20382	10 ml resin, 50 ml resin	4% agarose
	UltraLink® Immobilization Kit[1]	46500	5 x 2 ml columns + reagents	Polyacrylamide
	UltraLink® Biosupport Medium[1]	53112, 53110, 53111	2 ml resin, 10 ml resin, 50 ml resin	Polyacrylamide
	Reacti-Gel® (6X) Support	20259, 20260	10 ml resin, 50 ml resin	6% agarose
	Reacti-Gel® (GF-2000) Support	20377	50 ml resin	Trisacryl® GF-2000
Sulfhydryl-reactive	SulfoLink® Kit	44895	5 x 2 ml columns + reagents	6% agarose
	SulfoLink® Coupling Gel	20401, 20402	10 ml resin, 50 ml resin	6% agarose
	UltraLink® Iodoacetyl[1]	53155	10 ml resin	Polyacrylamide
Carbohydrate-reactive	CarboLink™ Kit	44900	5 x 2 ml columns + reagents	6% agarose
	CarboLink™ Coupling Gel	20391	10 ml resin	6% agarose
	UltraLink® Hydrazide	53149	10 ml resin	Polyacrylamide
Carboxyl-reactive	CarboxyLink™ Coupling Kit	44899	5 x 2 ml columns + reagents	4% agarose
	CarboxyLink™ Coupling Gel	20266	25 ml resin	4% agarose
	UltraLink® EDC/DADPA Immobilization Kit[1]	53154	5 x 2 ml columns + reagents	Polyacrylamide
Active hydrogen-reactive	PharmaLink™ Immobilization Kit	44930	5 x 2 ml columns + reagents	6% agarose
Antibody Orientation	ImmunoPure® rProtein A IgG Plus Orientation Kit	44893	2 x 2 ml columns + reagents	6% agarose
	ImmunoPure® Protein G IgG Plus Orientation Kit	44990	2 x 2 ml columns + reagents	6% agarose
GST Orientation	GST Orientation Kit	78201	2 x 2 ml columns + reagents	4% agarose
Fusion Protein Purification – Efficiently purify GST-, PolyHis- or MBP-tagged fusion proteins in a single step.				
GST-tagged	Immobilized Glutathione	15160	10 ml resin	4% agarose
	SwellGel® Immobilized Glutathione Kit[2]	89816	96-well plate + discs/reagents	Agarose
	SwellGel® Immobilized Glutathione Plate[2]	89815	96-well plate + discs	Agarose
	SwellGel® Immobilized Glutathione Discs[2]	89817	96 discs	Agarose
	B-PER® GST Fusion Protein Column Purification Kit	78200	5 x 1 ml columns + reagents	4% agarose
	B-PER® GST Fusion Protein Spin Purification Kit	78400	16 spin columns + reagents	4% agarose
	Y-PER® GST Fusion Protein Column Purification Kit	78997	5 x 1 ml columns + reagents	4% agarose
PolyHis-tagged	Nickel-Chelated Agarose	78320	8 ml resin	4% agarose
	SwellGel® Nickel Chelated Kit[2]	20147	96-well plate + discs/reagents	Agarose
	SwellGel® Nickel Chelated Plate[2]	75824	96-well plate + discs	Agarose
	SwellGel® Nickel Chelated Discs[2]	89827	96 discs	Agarose
	SwellGel® Cobalt Chelated Discs[2]	89838	96 discs	Agarose
	B-PER® 6xHis Fusion Protein Column Purification Kit	78100	5 x 1 ml columns + reagents	4% agarose
	B-PER® 6xHis Fusion Protein Spin Purification Kit	78300	16 spin columns + reagents	4% agarose
	Y-PER® 6xHis Fusion Protein Column Purification Kit	78994	5 x 1 ml columns + reagents	4% agarose
MBP-tagged	Immobilized Dextrin-10	15161	10 ml resin	4% agarose
Avidin-Biotin – Purify or immobilize biotinylated molecules through their interaction with avidin.				
Avidin	ImmunoPure® Immobilized Avidin	20219, 20225	5 ml resin, 25 ml resin	6% agarose
	AffinityPak™ Avidin Columns	20362	5 x 1 ml columns	6% agarose
Streptavidin	ImmunoPure® Immobilized Streptavidin	20347, 20349	2 ml resin, 5 ml resin	6% agarose
	AffinityPak™ Streptavidin Columns	20351	5 x 1 ml columns	6% agarose
	UltraLink® Immobilized Streptavidin[1]	53113, 53114	2 ml resin, 5 ml resin	Polyacrylamide
	UltraLink® Immobilized Streptavidin Plus[1]	53116, 53117	2 ml resin, 5 ml resin	Polyacrylamide
	MagnaBind™ Streptavidin Beads	21344	5 ml	Iron oxide
NeutrAvidin™ Biotin-Binding Protein	Immobilized NeutrAvidin™ on Agarose	29200	5 ml resin	6% agarose
	UltraLink® Immobilized NeutrAvidin™[1]	53150	5 ml resin	Polyacrylamide
	UltraLink® Immobilized NeutrAvidin™ Plus[1]	53151	5 ml resin	Polyacrylamide
Monomeric Avidin	ImmunoPure® Immobilized Monomeric Avidin	20228	5 ml resin	4% agarose
	ImmunoPure® Immobilized Monomeric Avidin Kit	20227	2 ml column + reagents	4% agarose
	UltraLink® Immobilized Monomeric Avidin[1]	53146	5 ml resin	Polyacrylamide
Biotin	Immobilized D-Biotin	20218	5 ml resin	6% agarose
	Immobilized Iminobiotin	20221	5 ml resin	6% agarose
Specialized Affinity Supports – Purify, or remove from solution, a variety of biologically important molecules.				
Phosphorylated Peptides	Phosphopeptide Isolation Kit	89853	30 isolations	Agarose
Met-containing Peptides	PrepTide™ Methionine Peptide Selective Matrix	21300	2 isolations	Porous glass
Trp-containing Peptides	PrepTide™ Tryptophan Peptide Selective Matrix	21310	2 isolations	Porous glass
Albumin	SwellGel® Blue Albumin Removal Kit[2]	89845, 89846	25 discs, 100 discs + reagents	Agarose
Heparin-binding Proteins	Immobilized Heparin Gel	20207	10 ml resin	4% agarose
C-reactive Protein	Immobilized P-Aminophenyl Phosphoryl Choline	20307	5 ml resin	6% agarose
Lectins	Immobilized D-Galactose	20372	5 ml resin	6% agarose
Glycoproteins	Immobilized Boronic Acid Gel	20244	10 ml resin	Polyacrylamide
His-tagged Proteins	Immobilized Iminodiacetic Acid	20277	10 ml resin	4% agarose
Proteases	Immobilized Pepstatin	20215	5 ml resin	6% agarose
	Immobilized Soybean Trypsin Inhibitor	20235	2 ml resin	4% agarose
Endotoxin	DetoxiGel™ AffinityPak™ Prepacked Columns	20344	5 x 1 ml columns	6% agarose
	DetoxiGel™ Endotoxin Removing Gel	20339, 20340	10 ml resin, 1,000 ml resin	6% agarose
Detergent	ExtractiGel® D Detergent Removing Gel	20208, 20303	10 ml resin, 100 ml resin	Proprietary
	ExtractiGel® D AffinityPak™ Columns	20346	5 x 1 ml columns	Proprietary

All products are available in bulk quantities upon request.

1. UltraLink® Support is a copolymer of polyacrylamide and azlactone with high surface area, large pore volume and low nonspecific binding. It is suitable for pressures up to 100 psi and linear flow rates up to 3,000 cm/hour.

Approximate Binding Capacity	Applications / Features
Range of 1-25 mg protein/ml resin 0.1-2 mg peptide/ml resin	Immobilize any protein through exposed lysine residues onto a rigid support for higher flow rates Stable, uncharged linkage for maximum binding specificity
Range of 1-20 mg protein/ml resin 0.1-2 mg peptide/ml resin	Immobilize any protein through exposed lysine residues Stable, uncharged linkage for maximum binding specificity
Range of 1-30 mg protein/ml resin 0.1-2 mg peptide/ml resin	Immobilize any protein through exposed lysine residues Rigid support for medium pressure applications
Range of 1-10 mg protein/ml resin 0.1-2 mg peptide/ml resin	Immobilize any protein through exposed lysine residues Stable, uncharged linkage for maximum binding specificity
Range of 1-10 mg protein/ml resin 0.1-2 mg peptide/ml resin	Immobilize peptides with a terminal cysteine residue for antibody purification Long spacer arm reduces steric hindrance for maximal antibody binding Immobilize proteins that contain free cysteine residues
Range of 1-5 mg glycoprotein/ml resin	Immobilize polyclonal antibodies and other glycoproteins Antibodies are oriented properly for maximum binding because attachment is through the Fc region
Range of 1-10 mg protein/ml resin 0.1-2 mg peptide/ml resin	Immobilize peptides/proteins through aspartic and glutamic acid residues or the carboxy terminus Long spacer arm reduces steric hindrance
Variable, up to 20 µmol/ml gel	Immobilize drugs and other organic molecules with no available reactive groups
Up to 16 mg rabbit IgG/column	Purify and covalently immobilize an antibody with a single column
Up to 16 mg rabbit IgG/column	
1-10 mg fusion protein/column	Purify and covalently immobilize GST fusion proteins with a single column
~10 mg fusion protein/ml resin	Purify GST-tagged fusion proteins
~1 mg fusion protein/well	
~1 mg fusion protein/well	
~1 mg fusion protein/disc	
~10 mg fusion protein/column	Purify GST-tagged fusion proteins
~1 mg fusion protein/spin column	Kit includes lysis reagent for optimal protein recovery
~10 mg fusion protein/column	
~10 mg fusion protein/ml resin	Purify His-tagged fusion proteins
~1 mg 6xHis-GFP/well	
~1 mg 6xHis-GFP/well	
>2 mg 6xHis-GFP/disc	
>2 mg 6xHis-GFP/disc	
~10 mg fusion protein/column	Purify His-tagged fusion proteins
~1 mg fusion protein/spin column	Kit includes lysis reagent for optimal protein recovery
~10 mg fusion protein/column	
~1.5 mg Dextrin-10/ml resin	Purify MBP-tagged fusion proteins
>20 µg biotin/ml resin	Isolate or immobilize biotinylated proteins, peptides, nucleic acids and other molecules
>20 µg biotin/ml resin	
1-3 mg biotinylated BSA/ml resin	Isolate or immobilize biotinylated proteins, peptides, nucleic acids and other molecules
1-3 mg biotinylated BSA/column	Gives lower background than avidin because it contains no carbohydrate
>2 mg biotinylated BSA/ml resin	
>4 mg biotinylated BSA/ml resin	
~2 µg biotin/ml resin	Magnetically isolate or immobilize biotinylated proteins, peptides, nucleic acids and other molecules
>20 µg biotin/ml resin	Isolate or immobilize biotinylated proteins, peptides, nucleic acids and other molecules
12-20 µg biotin/ml resin	Gives lowest background because carbohydrate has been removed and does not contain RYD sequence
>30 µg biotin/ml resin	
>1.2 mg biotinylated	BSA/ml resin Isolate or immobilize biotinylated proteins, peptides, nucleic acids and other molecules
>1.2 mg biotinylated BSA/ml resin	Reversible binding allows mild elution of biotinylated molecules
>1.2 mg biotinylated BSA/ml resin	
2 mg avidin/ml resin	Isolate or immobilize avidin molecules or conjugates
1 mg avidin/ml resin	Reversibly isolate avidin conjugates with mild elution conditions
150 µg phosphopeptide/isolation	Enrich phosphorylated peptides within a peptide digest for mass spectral analysis
~5 nmol protein digest Isolate	Methionine containing peptides to simplify mass spectral analysis
~2 nmol protein digest Isolate	Tryptophan containing peptides to simplify mass spectral analysis
~2 mg human serum albumin/disc	Remove albumin from antibodies and other samples
>0.2 mg heparin/ml resin	Purify a wide variety of proteins that have affinity for heparin
>3 mg human C-reactive protein/ml resin	Purify C-reactive protein
>8 mg castor bean lectin/ml resin	Purify lectins specific for D-galactose
100 µmol boronate/ml resin	Purify glycoproteins, ribonucleosides and other sugar-containing molecules
>14 µmol metal ions/ml resin	Purify His-tagged and other metal-binding proteins
1-2 mg pepsin/ml resin	Purify pepsin and cathepsins or remove them from a sample
Up to 6 mg trypsin/ml resin	Purify trypsin, chymotrypsin and elastase or remove them from a sample
2 mg endotoxin	Remove endotoxin from protein and nucleic acid samples
2 mg endotoxin/ml resin	
Varies among detergents - see page 56	Remove detergent from protein and nucleic acid samples
Varies among detergents - see page 56	

2. SwellGel® Support is a convenient, room temperature-stable, dehydrated agarose resin that is rapidly rehydrated when a sample is added. In a 96-well filterplate, SwellGel® Resin is ideal for high-throughput purifications.

PIERCE





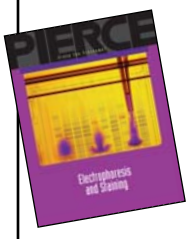
• Sample Prep • **Electrophoresis** • Markers • Staining • Blotting

Precisely our points

Introducing Precise™ Protein Gels.

- Easy-to-load lanes with durable plastic dividers
- Long shelf life – 12-month guarantee ensures consistent performance
- Unique running buffer for excellent separation and high-resolution protein bands
- Compatible with Laemmli sample buffer and standard mini-gel tanks

- Easy-to-open, no need for special tools
- Short run time – 45-minute run time provides results quickly
- 1 mm thick gels stain quickly and with high sensitivity using coomassie and silver stains
- Transfers quickly and efficiently to nitrocellulose and PVDF membranes for Western blotting



Precise™ Protein Gels are cast in a durable plastic cassette using a neutral pH buffer that prevents polyacrylamide breakdown. The gels are individually packaged in an easy-to-open plastic pouch and are ready to run with no comb or tape to remove. They are available in both gradient and fixed-concentrations and in 10-, 12- and 15-well formats. Visit our web site for the complete selection.

FREE! Electrophoresis and Staining Guide

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euroinfo@perbio.com	euroinfo@perbio.com	de.info@perbio.com	euroinfo@perbio.com	uk.info@perbio.com	euroinfo@perbio.com