

Antibody

Production

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Antibody Overview

Introduction to Antibody Production, Purification and Modification

Antibodies are host proteins that are produced by the immune system in response to foreign molecules that enter the body. These foreign molecules are called antigens, and their molecular recognition by the immune system results in selective production of antibodies that are able to bind the specific antigen. Antibodies are made by B lymphocytes and circulate throughout the blood and lymph where they bind to their specific antigen, enabling it to be cleared from circulation.

This ability of animal immune systems to produce antibodies capable of binding specifically to antigens can be harnessed to manufacture probes for detection of molecules of interest in a variety of research and diagnostic applications. No other current technology allows researchers to design and manufacture such highly specific molecular recognition tools. In addition to their high specificity, several important features make antibodies particularly conducive to development as probes. For example, except in those portions that determine antigen binding, antibodies share a relatively uniform and well-characterized protein structure that enables them to be purified, labeled and detected predictably and reproducibly by generalized methods.

Procedures for generating, purifying and modifying antibodies for use as antigen-specific probes were developed during the 1970s and 1980s and have remained relatively unchanged since Harlow and Lane published their classic *Antibodies: A Laboratory Manual* in 1988 (Product # 15050). Antibody production involves preparation of antigen samples and their safe injection into laboratory or farm animals so as to evoke high-expression levels of antigen-specific antibodies in the serum, which may then be recovered from the animal. Alternatively, monoclonal hybridoma cell lines that produce one particular antigen-specific antibody can be prepared by fusion of individual antibody-secreting spleen cells from immunized mice with immortal myeloma cell lines.

Antibody purification involves isolation of antibody from serum (polyclonal antibody), ascites fluid or culture supernatant of a hybridoma cell line (monoclonal antibody). Purification methods

range from very crude (precipitation of sample proteins including any antibodies present) to general (affinity purification of certain antibody classes without regard to antigen specificity) to specific (affinity purification of only those antibodies in a sample that bind to a particular antigen molecule). Which level of purification is necessary depends on the intended applications for the antibody.

Antibody characterization includes assessing antibody concentration and titer and determining the class and subclass of a purified antibody. Antibody concentration can be estimated by either a general protein assay or one of the species-specific Easy-Titer® IgG Assay Kits. Antibody titer refers to the functional dilution of an antibody sample necessary for detection in a given assay, such as an enzyme-linked immunosorbent assay (ELISA). Determining the class (e.g., IgG vs. IgM) and subclass (e.g., IgG₁ vs. IgG_{2a}) of an antibody is important for choosing an appropriate purification and modification method for the molecule. Class and subclass can be determined using an antibody isotyping kit (see page 22).

Purified antibodies may be modified for particular uses by several methods including fragmentation into smaller antigen-binding units, conjugation with enzyme or other detectable markers, and immobilization to solid supports. This handbook provides an overview of antibody structure and types, as well as of the procedures, reagents and tools used to produce, purify, fragment and label antibodies.

Structure of an Immunoglobulin Molecule

Antibody (or immunoglobulin) molecules are glycoproteins composed of one or more units, each containing four polypeptide chains: two identical heavy chains (H) and two identical light chains (L) (Figure 1). The amino terminal ends of the polypeptide chains show considerable variation in amino acid composition and are referred to as the variable (V) regions to distinguish them from the relatively constant (C) regions. Each L chain consists of one variable domain V_L and one constant domain C_L . The H chains consist of a variable domain, V_H , and three constant domains CH_1 , CH_2 and CH_3 . Each heavy chain has about twice the number of amino acids and MW (~50,000) as each light chain (~25,000), resulting in a total immunoglobulin MW of approximately 150,000.

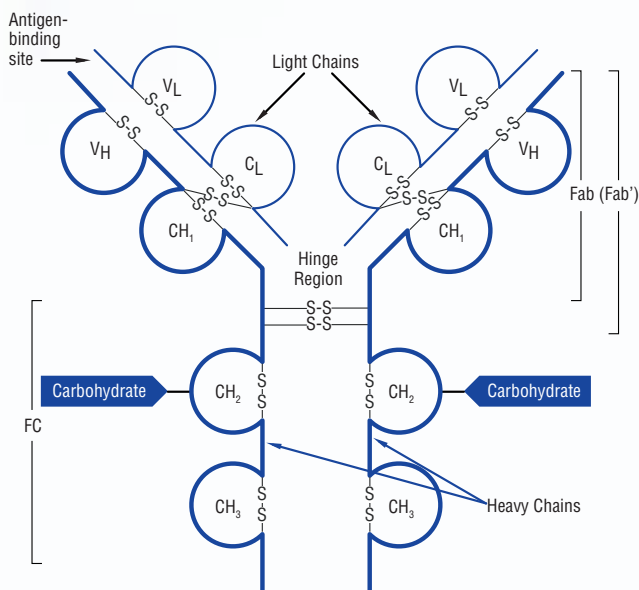


Figure 1. Structure of an immunoglobulin.

Heavy and light chains are held together by a combination of noncovalent interactions and covalent interchain disulfide bonds, forming a bilaterally symmetric structure. The V regions of H and L chains comprise the antigen-binding sites of the immunoglobulin (Ig) molecules. Each Ig monomer contains two antigen-binding sites and is said to be bivalent.¹

The hinge region is the area of the H chains between the first and second C region domains and is held together by disulfide bonds. This flexible hinge region allows the distance between the two antigen-binding sites to vary.²

Immunoglobulin Classes and Subclasses

The five primary classes of immunoglobulins are IgG, IgM, IgA, IgD and IgE. These are distinguished by the type of heavy chain found in the molecule. IgG molecules have heavy chains known as γ -chains; IgMs have μ -chains; IgAs have α -chains; IgEs have ϵ -chains; and IgDs have δ -chains. Differences in heavy chain polypeptides allow these immunoglobulins to function in different types of immune responses and at particular stages of the immune response. The polypeptide protein sequences responsible for these differences are found primarily in the Fc fragment. While there are five different types of heavy chains, there are only two main types of light chains: kappa (κ) and lambda (λ).

Antibody classes differ in valency as a result of different numbers of Y-like units (monomers) that join to form the complete protein. For example, in humans, IgM antibodies have five Y-shaped units (pentamer) containing a total of 10 light chains, 10 heavy chains and 10 antigen-binding sites.

IgG, a monomer, is the predominant Ig class present in human serum. Produced as part of the secondary immune response to an antigen, this class of immunoglobulin constitutes approximately 75% of total serum Ig. IgG is the only class of Ig that can cross the placenta in humans, and it is largely responsible for protection of the newborn during the first months of life.¹ Because of its relative abundance and excellent specificity toward antigens, IgG is the principle antibody used in immunological research and clinical diagnostics.

Serum IgM exists as a pentamer in mammals, predominates in primary immune responses to most antigens, is the most efficient complement-fixing immunoglobulin and constitutes approximately 10% of normal human serum Ig content. IgM is also expressed on the plasma membrane of the B lymphocytes as a monomer. It is the B cell antigen receptor, and the H chains each contain an additional hydrophobic domain for anchoring in the membrane. Monomers of serum IgM are bound together by disulfide bonds and a joining (J) chain.

Each of the five monomers is composed of two light chains (either κ or λ) and two heavy chains. Unlike in IgG, the heavy chain in IgM is composed of one variable and four constant domains with no hinge region. IgM can cause cell agglutination as a result of recognition of epitopes on invading microorganisms. This Ab/Ag immune complex is then destroyed by complement fixation or receptor-mediated endocytosis by macrophages.

IgA exists in serum in both monomeric and dimeric forms, constituting approximately 15% of the total serum Ig. Secretory IgA, a dimer, provides the primary defense mechanism against some local infections because of its abundance in membrane secretions (e.g., saliva, tears). The principal function of secretory IgA may not be to destroy antigen, but to prevent passage of foreign substances into the circulatory system.

IgD and IgE are found in serum in much smaller quantities than other Igs. Membrane IgD is a receptor for antigen found mostly on mature B lymphocytes. IgE primarily defends against parasitic invasion.

In addition to the major immunoglobulin classes, several Ig subclasses based on minor differences in heavy chain type of each Ig class exist in all members of a particular animal species. In humans there are four subclasses of IgG: IgG₁, IgG₂, IgG₃ and IgG₄ (numbered in order of decreasing concentration in serum). Variance between different subclasses is less than the variance between different classes. For example, IgG₁ is more closely related to IgG₂, IgG₃ or IgG₄ than to IgA, IgM, IgD or IgE. Consequently, there is general cross-reactivity among subclasses but very little cross-reactivity among different classes of Ig.

Polyclonal and Monoclonal Antibodies

Antibodies (whatever their class or subclass) are produced and purified in two basic forms for use as reagents in immunoassays: polyclonal and monoclonal. Typically, the immunological response to an antigen is heterogeneous, resulting in many different cell lines of B lymphocytes (precursors of plasma cells) producing antibodies to the same antigen. All of these cells originate from common stem cells, yet each develops the individual capacity to make an antibody that recognizes a particular determinant (epitope) on the same antigen. As a consequence of this heterogeneous response, serum from an immunized animal will contain numerous antigen-specific antibody clones, potentially of several different Ig classes and subclasses comprising generally 2-5% of the total Ig. Because it contains this heterogeneous collection of antigen-binding immunoglobulins, an antibody purified from such a sample is called a polyclonal antibody. Polyclonal antibodies are especially useful as labeled secondary antibodies in immunoassays.

Since an individual B lymphocyte produces and secretes only one specific antibody molecule, clones of B lymphocytes produce monoclonal antibodies. All antibodies secreted by a B cell clone are identical, providing a source of homogeneous antibody having a single defined specificity. However, while B lymphocytes can be isolated from suspensions of spleen or lymph node cells excised from immunized animals, they have a limited life span and cannot be cultured directly to produce antibody in useful amounts. Fortunately, this restriction has been overcome with the development of hybridoma technology, wherein isolated B lymphocytes in suspension are fused with myeloma cells from the same species (usually mouse) to create monoclonal hybrid cell lines that are virtually immortal while still retaining their antibody-producing abilities.³ Such hybridomas may be stored frozen and cultured as needed to produce the specific monoclonal antibody. Monoclonal antibodies are especially useful as primary antibodies in applications that require single-epitope specificity and an unchanging supply over many years of use. Hybridoma clones may be grown in cell culture for collection of antibodies from the supernatant or grown in the peritoneal cavity of a mouse for collection from ascitic fluid.






References

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2. Alberts, B., *et al.* (1983). Molecular Biology of the Cell. Garland Publishing, Inc., New York, NY.
3. Harlow, E. and Lane, D. (1988). Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. (Available from Pierce as Product # 15050.)

Table 1. Normal IgG concentrations from various sources.

Source	IgG concentration (approximate)
Ascites	2-10 mg/ml
Serum	10-16 mg/ml
Culture supernatant	0.5-1 mg/ml

Table 2. Important properties of antibody isotypes.

	IgG	IgM	IgA	IgE	IgD
Molecular weight	150,000	900,000	160,000 320,000 (secretory)	200,000	180,000
Heavy chain: Type	γ	μ	α	ϵ	δ
M.W.	53,000	65,000	55,000	73,000	70,000
Concentration in serum (approximate)	10-16 mg/ml	0.5-2 mg/ml	1-4 mg/ml	0.0001-0.0004 mg/ml	0-0.4 mg/ml
Percent of total IgG	80	6	13	0.002	0.2
Carbohydrate (approximate)	3%	12%	10%	12%	13%
Distribution	Intravascular and extravascular	Mostly intravascular	Intravascular and secretions	Basophils and mast cells in saliva and nasal secretions	Lymphocyte surface
Function	Secondary response	Primary response	Protect mucous membranes	Protect against parasites	Unknown
Structure					



Antibody Production

Overview

Production of antibodies is a relatively straightforward process involving immunization of animals and reliance on their immune systems to levy responses that result in manufacture of antibodies against the injected molecule.

However, because antibody production depends on such a complex biological system, results are not predictable; individual animals, even of the same genetic identity, will respond uniquely to the same immunization scheme, generating a different suite of specific antibodies against an injected molecule.

Nevertheless, equipped with a basic understanding of how the immune system responds to injection of a foreign substance and knowledge of available tools for preparing a sample for injection, a researcher can greatly increase the probability of obtaining a useful antibody product.

For example, chemical attachment of small compounds to carrier proteins makes it possible to induce what would otherwise be ineffective immune responses for production of specific antibodies. Pierce offers popular carrier proteins in both unmodified and preactivated forms ready for conjugation to test compounds. Expertise in cross-linking chemistry enables Pierce to offer activated carrier protein products of high quality and diverse reactive chemistries for conjugation to many different compounds. Adjuvants are substances that when mixed and injected with an immunogen increase the intensity of the immune response.

The Immune System

The immune system is a surveillance system designed to provide protection to its host from foreign invaders. The surveillance is mediated by proteins and cells that circulate throughout the organism to identify and destroy foreign cells, viruses or macromolecules.

Immune protection is provided by a dual system consisting of the cellular immune response and the humoral immune response. The cellular immune response is mediated by T lymphocytes and cannot be transferred from one individual to another by transfusion of serum. Humoral immunity involves soluble proteins found in serum (antibodies) that can be transferred to a recipient when serum is transfused.

Every cell in a vertebrate organism expresses the class I major histocompatibility complex (MHC I) on its plasma membrane. The MHC I presents endogenously derived peptide antigens to cytotoxic T lymphocytes (CTL). If the T cell receptor (TCR) of a CTL binds to the MHC I/peptide antigen on a cell, the entire cell is destroyed. This is a general description of the cellular immune response. The cellular immune response is targeted to intracellular pathogens such as viruses or bacteria (non-self) and cancer cells (altered self).

In contrast to the cellular response, the humoral response targets extracellular antigens. B lymphocytes use membrane IgM (mIgM) to bind antigen in its native form. Cross-linking of many mIgM and antigen molecules occurs (capping), and the complex is then taken into the cell by receptor-mediated endocytosis. This endosome then fuses with a lysosome and the resulting endolysosome digests the antigen into small peptides. The endolysosome fuses with a vesicle containing class II major histocompatibility complex (MHC II) molecules and the peptide antigens are bound by a cleft in the MHC II. This MHC II/antigen complex is then expressed on the plasma membrane of the B lymphocyte. The T cell receptor of a T helper lymphocyte then binds the MHC II/antigen and the T cell secretes cytokines, signaling the B lymphocyte to divide, differentiate and secrete antibodies. Without T help, the humoral response shuts down; in fact, the cellular response shuts down as well, as it does in AIDS.



Immunogenicity

Antigens and Immunogens

Successful generation of antibodies depends on the B lymphocyte binding, processing and presenting antigen to the T helper lymphocyte, which signals the B cell to produce and secrete antibodies. An antigen is any molecule that is identified as non-self by components of the immune system. An immunogen is an antigen that is able to evoke an immune response, including production of antibody via the humoral response. All immunogens are antigens, but not all antigens are immunogens. It is important to distinguish between the terms “antigen” and “immunogen” because many compounds are not immunogenic, and successful production of antibodies against such antigens requires that they be made immunogenic before injection by chemically attaching them to known immunogens.

Properties Determining Immunogenicity

Immunogenicity is the ability of a molecule to solicit an immune response. There are three characteristics that a substance must have to be immunogenic: foreignness, high molecular weight (MW) and chemical complexity. Foreignness is required so that the immunized animal does not recognize and ignore the substance as “self.” Generally, compounds from an organism are not immunogenic to that same individual and are only poorly immunogenic to others of the same or related species.

The second requirement for immunogenicity is high MW. Small compounds (i.e., with MW <1,000) such as penicillin, progesterone and aspirin, as well as many moderately sized molecules (i.e., with MW=1,000-6,000) are not immunogenic. Most compounds with a MW >6,000 are immunogenic. Compounds smaller than this can often be bound by mIgM on the surface of the B lymphocyte, but they are not large enough to facilitate cross-linking of the mIgM molecules. This cross-linking is commonly called “capping” and is the signal for receptor-mediated endocytosis of the antigen.

Finally, some degree of chemical complexity is required for a compound to be immunogenic. For example, even high MW homopolymers of amino acids and simple polysaccharides seldom make good immunogens because they lack the chemical complexity necessary to generate an immune response.

Macromolecules as Immunogens

It is possible to make certain generalizations about immunogenicity of the four major classes of macromolecules: carbohydrates, lipids, nucleic acids and proteins. Carbohydrates are immunogenic only if they have a relatively complex polysaccharide structure or form part of more complex molecules, such as glycoproteins.

Lipids usually are not immunogenic but can be made so by conjugation to a carrier protein. Likewise, nucleic acids are poor immunogens but can become immunogenic when coupled to a carrier protein.

Because of their complexity and size, proteins are generally strong immunogens. Given that most natural immunogens are macromolecules composed of protein, carbohydrate or a combination of the two, it is understandable that proteins are so broadly immunogenic. Peptides may have the complexity necessary to be immunogenic, but their small size usually renders them ineffective as immunogens on their own. Peptides are most often conjugated to carrier proteins to ensure that they induce an immune response and production of antibodies.

Haptens and Epitopes

Peptides and other small molecules that are used as antigens are referred to as haptens. They are antigenic, not immunogenic. Haptens can be made immunogenic by coupling to a suitable carrier molecule.

An epitope is the specific site on an antigen to which an antibody binds. For very small antigens, practically the entire chemical structure may act as a single epitope. Depending on its complexity and size, an antigen may cause production of antibodies directed at numerous epitopes. Polyclonal antibodies are mixtures of serum immunoglobulins and collectively are likely to bind to multiple epitopes on the antigen. Monoclonal antibodies by definition contain only a single antibody clone and have binding specificity for one particular epitope.

Specific antibodies can be generated against nearly any sufficiently unique chemical structure, either natural or synthetic, as long as the compound is presented to the immune system in a form that is immunogenic. The resulting antibodies may bind to epitopes composed of entire molecules (e.g., small haptens), particular functional groups of a larger molecule, unique arrangements of several amino acid functional groups in the tertiary structure of proteins, or any other unique structure in lipoproteins, glycoproteins, RNA, DNA or polysaccharides. Epitopes may also be parts of cellular structures, bacteria, fungi or viruses.

Carrier Proteins

A carrier protein is any protein used for coupling with peptides or other haptens that are not sufficiently large or complex on their own to induce an immune response and produce antibodies. The carrier protein, because it is large and complex, confers immunogenicity to the conjugated hapten, resulting in antibodies being produced against epitopes on the hapten and carrier. Many proteins can be used as carriers and are chosen based on immunogenicity, solubility and availability of useful functional groups through which conjugation with the hapten can be achieved. The two most commonly used carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA).

In a typical immune response, antibodies are produced by B lymphocytes (usually in conjunction with T-helper cells and antigen-presenting cells). In the majority of hapten-carrier systems, the B cells will produce antibodies that are specific for both the hapten and carrier. In the classically defined hapten-carrier system, T lymphocytes recognize processed carrier determinants and cooperate with B cells which induce a hapten-specific antibody response.

Since an antibody response will be directed against epitopes on both the carrier protein and hapten, it is important to plan carefully how hapten-specific antibodies will be identified and purified from the final immunized serum. To create the best immunogen, it may be beneficial to prepare the conjugates with several different carriers and with a range of hapten:carrier coupling ratios.

Keyhole Limpet Hemocyanin as Carrier Protein

Keyhole limpet hemocyanin, KLH, is widely used as a carrier protein because of its large molecular mass (4.5×10^5 - 8.0×10^6 kDa aggregates composed of 350 and 390 kDa subunits) and many available lysine groups. It is a copper-containing protein that belongs to a group of non-heme proteins called hemocyanins, which are found in arthropods and molluscs. Keyhole limpet hemocyanin is isolated from the mollusc *Megathura crenulata*. Divalent cations aid in the formation of large aggregates. Unlike with other gastropod hemocyanins, however, aggregates of KLH do not dissociate simply by removing divalent cations from the suspension.¹ While KLH exists in five different aggregate states in Tris buffer, pH 7.4, it reversibly dissociates to lower aggregate states or individual subunits with moderate changes in pH and completely dissociates at pH 8.9. Each subunit contains oxygen-binding sites, and one molecule of oxygen can be bound for every

two atoms of copper in KLH. The oxygen-containing protein is blue, while the oxygen-lacking form is colorless. Removal of oxygen also dissociates the protein to lower aggregate states. Increased antibody binding can be expected when KLH is dissociated into subunits because more antigenic sites will be available.²

Because of its size, KLH often suffers from poor water solubility. While this may not affect its immunogenicity, the handling of KLH in solution can be difficult. Even following removal of insoluble particles from a KLH solution, it is difficult to determine the amount of KLH present. KLH solutions are turbid, so 280 nm absorbance readings are inaccurate.

Imject® Mariculture Keyhole Limpet Hemocyanin from Pierce is purified and lyophilized in a stabilizing buffer. After reconstitution, the suspension-solution is an opalescent blue, which is characteristic of highly purified, nondenatured KLH. Pierce Imject® mKLH Products offer the combined advantages of high immunogenicity and good water solubility, making them ideal for hapten-carrier conjugation.

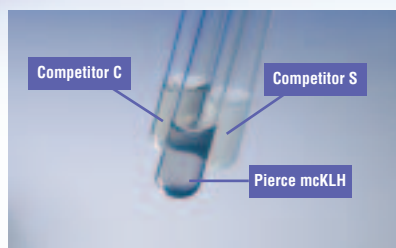
Traditionally, KLH was obtained from giant keyhole limpets harvested directly from the natural environment. This method disturbs the sensitive shoreline ecosystems where these limpets live. Current methods to obtain KLH are much less threatening to the natural habitat and limpet species survival. Giant keyhole limpets are raised in tanks and harvested (marine culture or "mariculture") where they are occasionally milked of some their fluids, similar to humans donating blood. They continue to live and thrive for many years.

Besides being obtained by a more environmentally friendly method, mKLH has some advantages over KLH as traditionally supplied. Most importantly for its use as a carrier protein, mKLH has improved uniformity and solubility, so that buffers containing high concentrations of sodium chloride are not necessary to prevent precipitation of the protein during hapten conjugation reactions and use. All Pierce KLH carrier protein products use mariculture KLH.

References

1. Sell, S. (1987). Immunology, Immunopathology, and Immunity. Elsevier, New York, NY.
2. Bartel, A. and Campbell, D. (1959). Arch. Biochem. Biophys. **82**, 2332.

Mariculture Keyhole Limpet Hemocyanin (mcKLH)



Highlights:

- Reformulated for enhanced solubility
- Elicits a stronger immune response than BSA or OVA
- Isolated from the mollusc *Megathura crenulata*
- Available lyophilized in PBS or MES buffer
- High molecular mass (4.5×10^5 to 8.0×10^6 MW)
- Numerous primary amines available for coupling haptens
- Environmentally friendly source

References

Cen, O., *et al.* (2003). *J. Biol. Chem.* **278**, 8837-8845.
 Herreman, A., *et al.* (2003). *J. Cell Sci.* **116**, 1127-1136.
 Jerry, D.J. (1993). *BioTechniques* **14**(3), 464-469.

Ordering Information

Product #	Description	Pkg. Size
77653	Imject® mcKLH (in MES buffer)	2 mg
77600	Imject® mcKLH (in PBS buffer)	5 x 20 mg
77649	Imject® mcKLH Subunits, High Purity	20 mg

Blue Carrier Immunogenic Protein

A solubilized, inexpensive alternative to KLH.

Blue Carrier Immunogenic Protein is a keyhole limpet hemocyanin (KLH)-like hemocyanin,¹ purified from the mollusc *Concholepas concholepas*. Mollusc hemocyanin, in general, is highly immunogenic due to its large size and distance from mammals along the phylogenetic tree. Blue Carrier Protein has been shown to be immunogenic² and is supplied already solubilized in a sterile, ready-to-conjugate PBS solution.

Highlights:

- Less expensive than KLH
- Pre-solubilized at 200 mg/ml in 0.5 ml of PBS buffer
- Provided sterile

References

1. Herscovitz, H.B., *et al.* (1972). *Immunology* **22**, 51-61.
 2. Becker, M.I., *et al.* (1998). *Hybridoma* **17**(4), 373-381.

Ordering Information

Product #	Description	Pkg. Size
77130	Blue Carrier Immunogenic Protein	100 mg

Bovine Serum Albumin and Ovalbumin as Carrier Proteins

Bovine serum albumin (BSA; MW 67,000) belongs to the class of serum proteins called albumins. Albumins constitute about half the protein content of plasma and are quite stable and soluble. BSA is much smaller than KLH but is nonetheless fully immunogenic. It is a popular carrier protein for weakly antigenic compounds. BSA exists as a single polypeptide with 59 lysine residues, 30-35 of which have primary amines that are capable of reacting with a conjugation reagent. Numerous carboxylate groups give BSA its net negative charge (pI 5.1). Imject® BSA is a highly purified (i.e., Fraction V) bovine serum albumin that, once reconstituted, can be used for conjugation to haptens without dialysis or further purification.

BSA is commonly used in development of immunoassays because it is readily available, is fully soluble and has numerous functional groups useful for cross-linking to small molecules that otherwise would not coat efficiently in polystyrene microplates. Furthermore, BSA is the most popular standard for protein assays, well-characterized as a molecular weight marker in SDS-PAGE and widely used as a blocking agent. These same characteristics that make BSA easy to use in immunoassay development also make it simple to use for preparing and testing conjugation efficiency of carrier-hapten conjugates. However, such multiple uses for BSA also require that steps be taken to avoid undesired cross-reactivity with the carrier in antibody-screening procedures and final applications.

Bovine Serum Albumin (BSA)

Highlights:

- Purified Fraction V BSA, lyophilized in either PBS or MES buffer
- MW 67 kDa
- Commonly used as a non-relevant carrier for ELISA analysis of antibody response

Reference

Harlow, E. and Lane, D. (1988). *Antibodies: A Laboratory Manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, pp. 56-100. (Product #15050) *This manual discusses the use of carrier proteins in detail.*

Ovalbumin (OVA)

Highlights:

- Often used as a non-relevant carrier protein in monoclonal screening ELISA assays
- Purified from hen egg whites
- Molecular weight is 45 kDa
- Available lyophilized in PBS or MES buffer
- Highly soluble in DMSO

For this reason, BSA is often used as a non-relevant protein carrier for antibody screening and immunoassays after using KLH as the carrier protein to generate the immune response against the hapten. Only by using different carrier proteins in the immunization and screening/purification steps can one be assured of detecting hapten-specific rather than carrier-specific antibodies. Using BSA as the non-relevant carrier protein generally allows one to take greater advantage of its properties as standard, MW marker and blocking agent.

Ovalbumin (OVA; MW 45,000) can be used as a carrier protein. Also known as egg albumin, ovalbumin constitutes 75% of protein in hen egg whites. OVA contains 20 lysine groups and is most often used as a secondary (screening) carrier rather than for immunization, although it is immunogenic. The protein also contains 14 aspartic acid and 33 glutamic acid residues that afford carboxyl groups. These may be utilized for conjugation to haptens. Ovalbumin exists as a single polypeptide chain having many hydrophobic residues and an isoelectric point of 4.63. The protein denatures at temperatures above 56°C or when subject to electric current or vigorous shaking. OVA is unusual among proteins in being soluble in high concentrations of the organic solvent DMSO, enabling conjugation to haptens that are not easily soluble in aqueous buffers.

Ordering Information

Product #	Description	Pkg. Size
77171	Imject® Bovine Serum Albumin (in MES buffer)	2 mg
77110	Imject® Bovine Serum Albumin (in PBS buffer)	5 x 20 mg

Reference

Harlow, E. and Lane, D. (1988). *Antibodies: A Laboratory Manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, pp. 56-100. (Product #15050) *This manual discusses the use of carrier proteins in detail.*

Ordering Information

Product #	Description	Pkg. Size
77109	Imject® Ovalbumin (in MES buffer)	2 mg
77120	Imject® Ovalbumin (in PBS buffer)	5 x 20 mg

SuperCarrier® Immune Modulator and SuperCarrier® Systems

Considerable immunological research has focused on understanding the nature of antigen recognition and the cellular interactions involved in the generation and regulation of the immune response. In one study, BSA was modified by substituting anionic carboxyl groups with cationic aminoethylamide groups (Figure 2).¹ This cationized BSA (cBSA) resulted in an immunogen that stimulates a much higher antibody response than the native form of the molecule. *In vivo*, the antibody response is not only increased, but remains elevated for an extended period of time. *In vitro*, much less cBSA than native BSA is required to produce the same degree of T cell proliferation. Although it is modified, cBSA retains most of the immunogenic determinants of native BSA.

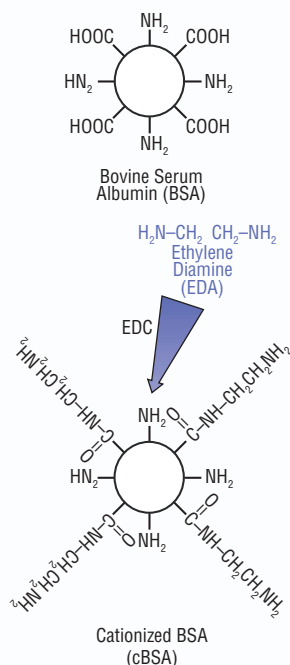


Figure 2. Preparation of cationized BSA (SuperCarrier® Immune Modulator).

The cationized immunogen also exhibits altered regulatory properties. Intravenous or oral administration of cBSA to mice prior to intraperitoneal challenge results in immunogen-specific enhancement of the response rather than suppression; this is observed with intravenous or oral native BSA pre-treatment.¹ The underlying mechanism is related to the form of immunogen that is recognized by the various cells regulating the response. Because of its net positive charge (pI >11), cBSA has a greater affinity for the negatively charged cell surface membrane of the antigen-presenting cell. Internalization of cBSA occurs by receptor-mediated endocytosis, an adsorptive uptake mechanism, rather

than by pinocytosis.² This results in more efficient uptake and processing of the immunogen into a more immunogenic form.

Interestingly, the immune response enhancement caused by cBSA extends to haptens or other proteins to which it is conjugated. For example, when used to immunize mice, ovalbumin conjugated to cBSA elicits greater anti-ovalbumin antibody production than ovalbumin alone or ovalbumin-BSA conjugate (Figure 3).

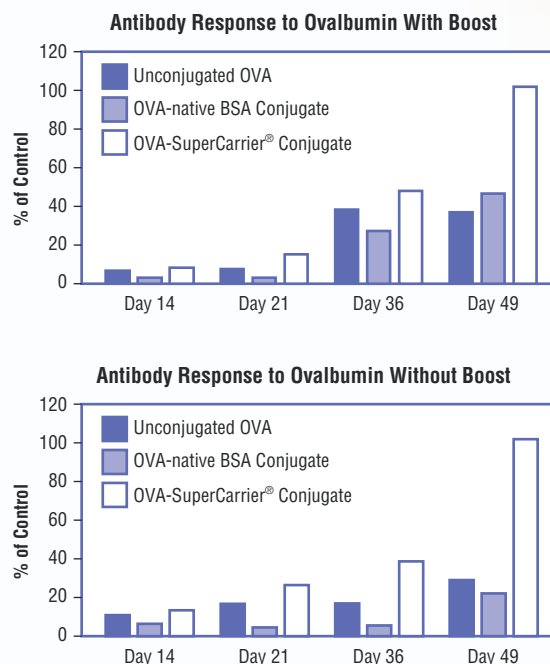


Figure 3. Antibody response to ovalbumin.

Pierce offers SuperCarrier® Immune Modulator for use with both haptens and full-sized protein antigens to elicit an enhanced immunological response toward a coupled molecule. The use of cBSA as a carrier for both proteins and peptides eliminates the need to develop protocols for each antigen system.

SuperCarrier® Immune Modulator will produce an anti-peptide response greater than that seen with a traditional carrier and is effective for enhancing the antibody response to proteins with low pI. Native charge, rather than the size of the protein, appears to be a more important determinant of the potential for enhancement using SuperCarrier® Immune Modulator. There may be additional factors that influence the effectiveness of the conjugate.

References

1. Muckerheide, A., et al. (1987). *J. Immunol.* **138**, 833-837.
2. Apple, R.J., et al. (1988). *J. Immunol.* **140**, 3290-3295.

SuperCarrier® Immune Modulator

Pierce SuperCarrier® Immune Modulator will enhance the antibody response to large proteins as well as haptens conjugated to it. Native OVA generates a lower antibody titer than OVA conjugated to SuperCarrier® Immune Modulator (Figure 3, previous page). The SuperCarrier® System is so potent that a second booster immunization may not be necessary.

Highlights:

- Unique cationized BSA carrier enhances immune response for both haptens and large proteins
- Stronger immune response than BSA or OVA; no need for Freund's Complete Adjuvant
- Enhanced antibody response of long duration
- Does not aggregate during EDC conjugation
- So potent that a second booster immunization may not be necessary

References

- Briggs, S.D., *et al.* (2001). *Genes Dev.* **15**, 3286-3295.
 Domen, P.L., *et al.* (1987). *J. Immunol.* **139**, 3195-3198.
 Hachiya, A., *et al.* (2002). *J. Biol. Chem.* **277**, 5395-5403.
 Muckerheide, A., *et al.* (1987). *J. Immunol.* **138**, 833-837.
 Muckerheide, A., *et al.* (1987). *J. Immunol.* **138**, 2800-2804.

Ordering Information

Product #	Description	Pkg. Size
77165	Imject® SuperCarrier® Immune Modulator* (in MES buffer)	2 mg
77150	Imject® SuperCarrier® Immune Modulator* (in PBS buffer)	10 mg

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

Hapten-Carrier Conjugation

Several approaches are available for conjugating haptens to carrier proteins. The choice of which conjugation chemistry to use depends on the functional groups available on the hapten, the required hapten orientation and distance from the carrier, and the possible effect of conjugation on biological and antigenic properties. For example, proteins and peptides have primary amines (the N-terminus and the side chain of lysine residues), carboxylic groups (C-terminus or the side chain of aspartic acid and glutamic acid) and sulfhydryls (side chain of cysteine residues) that can be targeted for conjugation. Generally, it is the many primary amines in a carrier protein that are used to couple haptens via a cross-linking reagent of one kind or another.

Carboxyl-to-Amine Conjugation Using EDC

Because most proteins contain both exposed lysines and carboxyl groups, EDC (Product # 22980, 22981)-mediated immunogen formation may be the simplest method for the majority of protein-carrier conjugations. The carbodiimide initially reacts with available carboxyl groups on either the protein carrier or peptide hapten to form an active O-acylurea intermediate (Figure 4). This intermediate then reacts with a primary amine to form an amide bond and release of a soluble urea derivative. This efficient reaction produces a conjugated immunogen in less than two hours.

Like most immunogen coupling reagents, EDC is subject to hydrolysis and should be protected from moisture until used. The hydrolysis of EDC is a competing reaction during coupling and is dependent on temperature, pH and buffer concentration.¹ In general, EDC coupling is a very efficient, one-step method for forming a wide variety of protein-carrier and peptide-carrier immunogens. Conjugation may occur at any carboxyl or primary amine-containing amino acid side chains; therefore, this method should be avoided if the antigenic sites of interest in the protein or peptide contain groups that may be blocked or undergo coupling from the carbodiimide reaction.

In general, conjugations mediated by EDC result in considerable polymerization when proteinaceous antigens and carriers are involved. This occurs because most peptides and antigens contain both primary amines and carboxylates (at least in their N- and C-termini, respectively). Some peptides will conjugate to themselves (end-to-end by their N- and C- termini or through side chains) as well as to the carrier protein. Likewise, the carrier protein will conjugate to itself. Such polymerization is not necessarily a disadvantage for immunogenicity and antibody production, but large polymers can decrease the solubility of the conjugate, making its subsequent handling and use more difficult. Some polymerized peptide on the surface of the carrier may actually enhance the immunogenicity of the peptide, effecting a greater antibody response. SuperCarrier® Immune Modulator is practically devoid of carboxylic groups, so the possibility of carrier polymerization is minimized in EDC conjugations with this carrier protein.

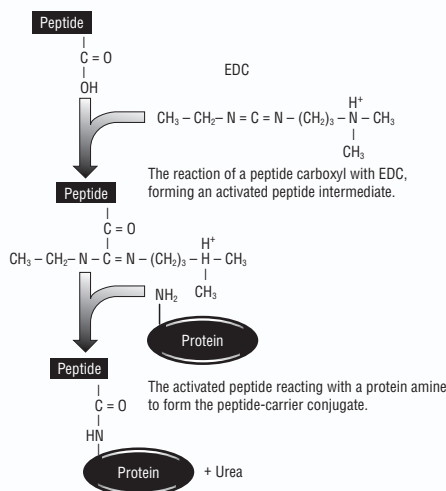


Figure 4. EDC-mediated hapten-carrier conjugation.

Reference

1. Bartel, A. and Campbell, D. (1959). *Arch. Biochem. Biophys.* **82**, 2332.

Conjugation Through Sulfhydryl Groups (Reduced Cysteines)

A peptide synthesized with a terminal cysteine residue has a sulfhydryl group that provides a highly specific conjugation site for reacting with certain cross-linkers. For example, the heterobifunctional cross-linker Sulfo-SMCC (Product # 22322) contains a maleimide group that will react with free sulfhydryls and a succinimidyl (NHS-ester) group that will react with primary amines. By reacting the reagent first to the carrier protein (with its numerous amines) and then to a peptide containing a reduced terminal cysteine, all peptide molecules will be coupled with the same predictable orientation (Figure 5).

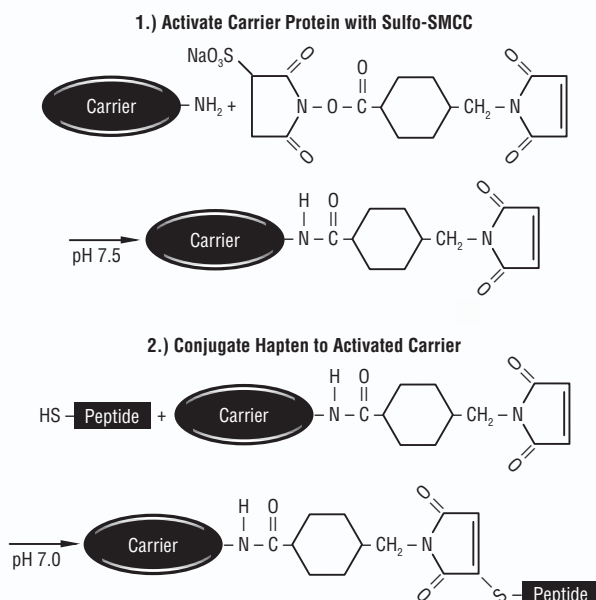


Figure 5. Hapten-Carrier conjugation with the heterobifunctional cross-linker Sulfo-SMCC.

The carrier is first activated by conjugating to the active ester end of Sulfo-SMCC via amino groups above pH 7.0. This reaction results in the formation of an amide bond between the protein and the cross-linker, with the release of Sulfo-NHS as a byproduct. The carrier protein is then isolated by gel filtration to remove excess reagents. At this stage, the purified carrier possesses

modifications generated by the cross-linker, resulting in a number of reactive maleimide groups projecting from its surface. The maleimide group of Sulfo-SMCC is stable for hours in solution at a physiological pH. Therefore, even after the activation and purification steps, the greatest possible activity will remain for conjugation with a peptide. The maleimide group of Sulfo-SMCC reacts at pH 6.5-7.5 with free sulfhydryls on the peptide to form a stable thioether bond.

Sulfo-SMCC is subject to hydrolysis and should be kept away from moisture. The reaction of carrier and protein is very efficient and requires only three hours for the preparation of a conjugated immunogen. Pierce also offers SMCC (Product # 22360), the nonwater-soluble version of Sulfo-SMCC, as well as several other related heterobifunctional cross-linkers. The water solubility of Sulfo-SMCC, along with its enhanced maleimide stability, makes it a favorite for hapten-carrier conjugation.

Maleimide-Activated Carrier Proteins

Imject® Maleimide Activated Carrier Proteins are commonly used carrier proteins that have been pre-activated with the heterobifunctional cross-linker Sulfo-SMCC, gel filtered and lyophilized to make them ready for direct conjugation to sulfhydryl-containing haptens. In two hours a hapten-carrier conjugate is formed via a stable thioether bond. The activation reaction is the more difficult step in Sulfo-SMCC conjugations between molecules (Figure 5, step 1). For example, the unreacted cross-linker is much more susceptible to hydrolysis than the intermediate activated protein. Imject® Maleimide Activated Carrier Proteins include mCKLH, BSA, OVA and SuperCarrier® Immune Modulator and are available in 2 mg and 10 mg sizes. These quality-tested, pre-activated carrier proteins save precious research time and ensure reproducible results because they are activated to known levels (Table 3).

Table 3. Activation levels of Imject® Maleimide Activated Carrier Proteins.

Carrier	Maleimide Groups per molecule of carrier	Molecular Weight (unactivated carrier)
Imject® Activated mCKLH	>100	~ 8.0 × 10 ⁶
Imject® Activated OVA	5-15	45,000
Imject® Activated BSA and Super Carrier®	15-25	67,000

Hapten-carrier conjugations using Sulfo-SMCC or Imject® Maleimide Activated Carrier Proteins require that the hapten (commonly a peptide) contain a free sulfhydryl group. Some peptides already have cysteines in their sequence, while others are appended with a terminal cysteine during synthesis. Such peptides usually dimerize through the formation of disulfide bridges between cysteines, preventing conjugation to maleimide-activated carrier proteins. Therefore, unless otherwise known to have free sulfhydryls, disulfide bridges in cysteine-containing peptides and proteins should be reduced before conjugation. Reducing agents such as Dithiothreitol (DTT), 2-Mercaptoethanol (2-ME) or TCEP (Product #s 20290, 35600 and 20490, respectively) are commonly used for this purpose. Immobilized TCEP (Product # 77712) allows reduction of disulfides without the problems associated with subsequent separation of reductant from reduced peptide or protein, which is required for conjugation with maleimide-activated carriers.

Haptens that do not contain sulfhydryls (e.g., peptides without cysteines) can be modified at primary amines to introduce sulfhydryls using Traut's Reagent (Product # 26101) or SATA (Product # 26102). SATA introduces a protected sulfhydryl group that can be exposed (i.e., made available for use) with Hydroxylamine•HCl (Product # 26103). The Protein-Coupling Handle Addition Kit (Product # 23460) contains SATA and hydroxylamine in a convenient kit to add sulfhydryl groups to a protein. Although this strategy effects a linkage through primary amines on both hapten and carrier protein, more control over the conjugation is possible in this two-stage procedure than with direct amine-to-amine conjugation using a homobifunctional cross-linker such as DSS (Product # 21555).

Imject® Maleimide Activated Carrier Proteins

Immunogens made the fast and easy way!

Imject® Maleimide Activated Carrier Proteins are commonly used carriers that have been preactivated with a heterobifunctional cross-linker (Sulfo-SMCC). This activation results in a stable, sulfhydryl-reactive carrier protein.

Highlights:

- Save time – no need to preactivate the carrier with Sulfo-SMCC
- Conjugation can be performed directly in the vial (because of EDTA in the buffer, gel filtration or dialysis must be performed prior to immunization)
- A hapten-carrier conjugate is formed in just two hours
- Results in a stable, covalent thioether linkage
- Purified proteins, activated with sulfhydryl-reactive maleimide groups
- Lyophilized in sodium phosphate, EDTA, NaCl Buffer, pH 7.2, plus stabilizers

References for Maleimide-Activated KLH

Iijima, N., *et al.* (2003). *Eur. J. Biochem.* **270**, 675-686.
 Rexer, B.N. and Ong, D.E. (2002). *Biol. Reprod.* **67**, 1555-1564.
 Wang, Z., *et al.* (2002). *J. Biol. Chem.* **277**, 24022-24029.
 Ellison, V. and Stillman, B. (2003). *PLOS Biology* **1**, e33.
 Young, L., *et al.* (2001). *Science* **291**, 2135-2138.

Reference for Maleimide-Activated SuperCarrier® Protein

Borge, P.B. and Wolf, B.A. (2003). *J. Biol. Chem.* **278**, 11359-11368.

References for Maleimide-Activated BSA

Buscaglia, C.A., *et al.* (2003). *Mol. Biol. Cell* **14**, 4947-4957.
 Foletti, D.L. and Scheller, R.H. (2001). *J. Neurosci.* **21**, 5461-5472.

Reference for Maleimide-Activated Ovalbumin

Bomont, P. and Koenig, M. (2003). *Hum. Mol. Genet.* **12**, 813-822.

Ordering Information		
Product #	Description	Pkg. Size
77606	Imject® Maleimide Activated Mariculture Keyhole Limpet Hemocyanin (mcKLH)	2 mg
77605	Imject® Maleimide Activated Mariculture Keyhole Limpet Hemocyanin (mcKLH)	10 mg
77610	Imject® Maleimide Activated Mariculture Keyhole Limpet Hemocyanin (mcKLH)	10 x 10 mg
77175	Imject® Maleimide Activated SuperCarrier® Immune Modulator	2 mg
77155	Imject® Maleimide Activated SuperCarrier® Immune Modulator	10 mg
77116	Imject® Maleimide Activated Bovine Serum Albumin	2 mg
77115	Imject® Maleimide Activated Bovine Serum Albumin	10 mg
77126	Imject® Maleimide Activated Ovalbumin	2 mg
77125	Imject® Maleimide Activated Ovalbumin	10 mg
77164	Imject® Maleimide Conjugation Buffer	30 ml
77159	Imject® Purification Buffer Salts (For use with mcKLH)	5 g
22322	Sulfo-SMCC (Sulfosuccinimidyl 4-[N-maleimidomethyl]-cyclohexane-1-carboxylate)	50 mg
22360	SMCC (Succinimidyl 4-[N-maleimidomethyl]-cyclohexane-1-carboxylate)	50 mg

Immunogen Conjugation Kits

Pierce offers a line of Imject® Immunogen Conjugation Kits that include complete sets of reagents, carrier proteins and step-by-step instructions for efficient preparation of hapten-carrier conjugates for production of hapten-specific antibodies. Successful hapten-carrier protein conjugation protocols can take time to develop because there are many variables to consider, including choice of carrier protein, coupling reagents, reaction conditions and readiness of the conjugate for injection into an animal. Not only must reagents be obtained or prepared but also procedures must be in place to verify that the conjugation was successful and to clearly assay production of hapten-specific antibodies.

The Imject® Maleimide Activated Immunogen Conjugation Kits (page 14) feature Imject® Maleimide Activated Carrier Proteins. These exclusive, activated proteins react with sulfhydryl-containing peptides in two hours, forming stable, covalently coupled hapten-carrier conjugates. Most of these Immunogen Kits contain two activated carrier proteins. In addition, each kit contains conjugation and purification buffers, and desalting columns for purifying the conjugate. Instructions for kits also describe methods to estimate the number of haptens coupled to the carrier protein prior to immunization. One of these methods uses Ellman's Reagent (5,5'-dithio-*bis*-[2-nitrobenzoic acid], Product # 22582), which reacts with free sulfhydryls to form a highly colored chromophore with an absorbance maximum at

412 nm. After the conjugation step, any unreacted hapten is measured. This is compared with the amount of starting hapten to determine the quantity of hapten conjugated to protein.

Imject® Immunogen EDC Conjugation Kits (page 15) provide reagents, optimized reaction conditions and complete instructions to assure successful conjugation of haptens through carboxyl groups. Most of these Imject® Immunogen EDC Conjugation Kits contain two carrier proteins, mcKLH or SuperCarrier® Immune Modulator, to use for immunization and another to use as a non-relevant carrier in screening for hapten-specific antibodies. Conjugates with the non-relevant carrier are ideal for coating microplates to facilitate hapten adsorption and eliminate errors due to carrier-specific antibody binding. Besides the one or two carrier proteins and the cross-linker EDC, these kits include conjugation and purification buffers and desalting columns for purifying the conjugate to make it ready for injection.

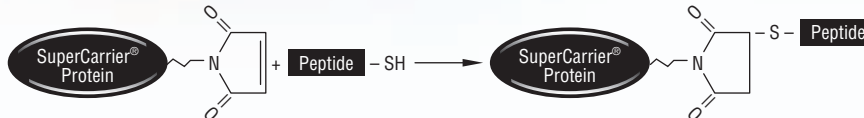
The PharmaLink™ Immunogen Kit (page 16) includes Imject® SuperCarrier® Immune Modulator and all reagents necessary for preparing immunogens of small haptens lacking the usual functional groups that can be targeted for conjugation. As long as the hapten (e.g., small metabolites or drug molecule) contains active hydrogens in its structure, the Mannich Reaction used in this kit will be successful in making the hapten-carrier conjugate.

Imject® Maleimide Activated Immunogen Conjugation Kits

Include Imject® Activated Carrier Proteins and everything needed for easy conjugations.

Hapten Conjugation to Imject® Maleimide Activated SuperCarrier® Protein

The reaction of Maleimide Activated SuperCarrier® Immune Modulator with peptide sulfhydryl, forming a stable thioether bond between hapten and SuperCarrier® Protein.



Pierce Imject® Activated Immunogen Kits save time when producing immunogens. The maleimide chemistry provides a convenient way to couple sulfhydryl-containing compounds to the carrier. Kits are supplied with or without non-relevant carrier proteins to screen for antibody titer against the antigen and not the carrier protein.

For example, a peptide with a terminal cysteine residue can be conjugated to Maleimide Activated mKLH. This peptide-mKLH conjugate is then used to immunize mice. To screen for specific anti-peptide antibodies from serum, couple the peptide to the second carrier (Maleimide Activated OVA) and use this conjugate as the antigen on the ELISA plate. This allows identification of only anti-peptide antibodies and not anti-carrier (mKLH) antibodies. The figure above shows how maleimide-activated cBSA (SuperCarrier® Cationized BSA) reacts with a sulfhydryl-containing hapten.

It is also possible to isolate anti-hapten antibodies without co-purifying anti-carrier protein antibodies. By using the same conjugation chemistry to immobilize the hapten on a beaded support, a specific affinity column can be created to purify anti-hapten antibodies. If using the Imject® Maleimide Activated Immunogen Kit to generate antibodies, use a Pierce SulfoLink® Kit (Product #s 44895, 20405) to immobilize the hapten and purify anti-hapten antibodies.

Highlights:

- Eliminate the development work necessary to produce hapten-carrier conjugates
- Maleimide-activated carrier proteins react with sulfhydryl-containing peptides
- Form stable hapten-carrier conjugates in just two hours
- Complete kits are available with or without irrelevant carriers
- Sufficient reagents for five separate conjugations

References

- Löhr, C.V., *et al.* (2002). *Infect. Immun.* **70**, 6005-6012.
 Madigan, J.P., *et al.* (2002). *Nucleic Acids Res.* **30**, 3698-3705.
 Moreno, C.S., *et al.* (2000). *J. Biol. Chem.* **275**, 5257-5263.
 Plager, D.A., *et al.* (1999). *J. Biol. Chem.* **274**, 14464-14473.
 Schober, J.M., *et al.* (2003). *J. Biol. Chem.* **278**, 25808-25815.

Ordering Information

Product #	Description	Pkg. Size
77611	Imject® Maleimide Activated mKLH Kit Includes: Imject® Maleimide Activated mKLH All Conjugation and Purification Buffers Desalting Columns	Kit 5 x 2 mg 5 x 5 ml
77607	Imject® Maleimide Activated Immunogen Conjugation Kit with mKLH and BSA Includes: Imject® Maleimide Activated mKLH Imject® Maleimide Activated BSA Conjugation Buffer Purification Buffer Salts Desalting Columns	Kit 5 x 2 mg 5 x 2 mg 30 ml 5 x 5 g 10 x 5 ml
77112	Imject® Maleimide Activated BSA Kit Includes: Imject® Maleimide Activated BSA All Conjugation and Purification Buffers Desalting Columns	Kit 5 x 2 mg 5 x 5 ml
77608	Imject® Maleimide Activated Immunogen Conjugation Kit with mKLH and OVA Includes: Imject® Maleimide Activated mKLH Imject® Maleimide Activated OVA Conjugation Buffer Purification Buffer Salts Desalting Columns	Kit 5 x 2 mg 5 x 2 mg 30 ml 5 x 5 g 10 x 5 ml
77113	Imject® Maleimide Activated OVA Kit Includes: Imject® Maleimide Activated OVA All Conjugation and Purification Buffers Desalting Columns	Kit 5 x 2 mg 5 x 5 ml
77656	Imject® Maleimide Activated SuperCarrier® Kit with Super Carrier® Immune Modulator and mKLH Includes: Imject® Maleimide Activated SuperCarrier® Immune Modulator* Imject® Maleimide Activated mKLH Conjugation Buffer Purification Buffer Salts Desalting Columns Imject® Alum	Kit 5 x 2 mg 5 x 2 mg 30 ml 5 x 5 g 10 x 5 ml 50 ml

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

Imject® Immunogen EDC Conjugation Kits

EDC conjugates amines to carboxyls for guaranteed hapten-carrier conjugation.

Because most proteins contain both exposed lysines and carboxyl groups, EDC (Product # 22980, 22981)-mediated immunogen formation may be the simplest method for the majority of protein-carrier conjugations. The reactions involved in an EDC conjugation are shown in Figure 4.

In general, EDC coupling is a very efficient, one-step method for forming a wide variety of immunogens. Conjugation may occur at any carboxyl- or primary amine-containing side chain. Therefore, this method should be avoided if the area of interest in the protein contains groups that may be blocked or undergo coupling from the carbodiimide reaction.

Highlights:

- Eliminate the development work necessary to produce effective hapten-carrier conjugates and anti-hapten antibodies
- Each kit contains sufficient materials to immunize 10 rabbits or 30 mice
- Complete kits now available with or without irrelevant carriers for performing screening assays
- Sufficient reagents for five separate conjugations

You can also isolate anti-hapten antibodies without co-purifying anti-carrier protein antibodies. By using the same conjugation chemistry to immobilize the hapten on a beaded support, a specific affinity column can be created to purify anti-hapten antibodies. If using the Imject® Immunogen EDC Conjugation Kits to generate antibodies, then use the CarboxyLink™ Kit (Product # 44899) to immobilize the hapten and purify anti-hapten antibodies.

References

- del Arco, A., *et al.* (2002). *Eur. J. Biochem.* **269**, 3313-3320.
Horiuchi, J., *et al.* (2004). *J. Biol. Chem.* **279**, 12117-12125.
Shanks, R.A., *et al.* (2002). *J. Biol. Chem.* **277**, 40967-40972.
Steadman, B.T., *et al.* (2002). *J. Biol. Chem.* **277**, 30165-30176.

Ordering Information

Product #	Description	Pkg. Size	U.S. Price
77622	Imject® Immunogen EDC Kit with mcKLH Includes: mcKLH EDC All Conjugation and Purification Buffers Desalting Columns	Kit 5 x 2 mg 5 x 10 mg 5 x 5 ml	\$300
77601	Imject® Immunogen EDC Kit with mcKLH and BSA Includes: mcKLH BSA EDC Conjugation Buffer Desalting Columns Purification Buffer Salts	Kit 5 x 2 mg 5 x 2 mg 10 x 10 mg 30 ml 10 x 5 ml 5 x 5 g	
77123	Imject® Immunogen EDC Kit with BSA Includes: BSA EDC All Conjugation and Purification Buffers Desalting Columns	Kit 5 x 2 mg 5 x 10 mg 5 x 5 ml	
77602	Imject® Immunogen EDC Kit with mcKLH and OVA Includes: mcKLH OVA EDC Conjugation Buffer Desalting Columns Purification Buffer Salts	Kit 5 x 2 mg 5 x 2 mg 10 x 10 mg 30 ml 10 x 5 ml 5 x 5 g	
77124	Imject® Immunogen EDC Kit with OVA Includes: OVA EDC All Conjugation and Purification Buffers Desalting Columns	Kit 5 x 2 mg 5 x 10 mg 5 x 5 ml	
77652	Imject® SuperCarrier® EDC Kit for Peptides Includes: SuperCarrier® Immune Modulator* mcKLH EDC Purification Buffer Salts Conjugation Buffer Desalting Columns Imject® Alum	Kit 5 x 2 mg 5 x 2 mg 10 x 10 mg 5 x 5 g 30 ml 10 x 5 ml 50 ml	
77151	Imject® SuperCarrier® EDC Kit for Proteins Includes: SuperCarrier® Immune Modulator* EDC Purification Buffer Salts Conjugation Buffer Desalting Columns Imject® Alum	Kit 5 x 2 mg 5 x 10 mg 5 x 5 g 30 ml 5 x 5 ml 50 ml	
77162	Imject® EDC Conjugation Buffer	30 ml	

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

Other Hapten-Carrier Conjugation Chemistries

Haptens, including many drugs, steroids and polysaccharides, do not contain amines, carboxylates or sulfhydryls. Yet, conjugation to a carrier protein is necessary if they are to be made immunogenic and allow production of antibody.

In theory, any of the cross-linking and modification reagents offered by Pierce can be used to prepare hapten-carrier protein conjugates (consult the Protein Structure section of the Pierce Technical Handbook and Catalog for more details). For example, haptens containing sugar groups or polysaccharide chains can be conjugated by reductive amination to primary amines on carrier proteins. The reaction requires that diols in sugar rings be oxidized to active aldehydes, which will react to primary amines.

Any molecule that has an active hydrogen can be conjugated to primary amines in the presence of formaldehyde, a scheme known as the Mannich Reaction (Figure 6). A kit of reagents for using this approach to conjugate small haptens to Imject® SuperCarrier® Immune Modulator are provided in the PharmaLink™ Immunogen Kit.

When choosing a conjugation chemistry for preparation of an immunogen, care must be taken to prevent altering the hapten too much or the antibodies raised against its epitopes will not recognize the native target molecule. For peptides, careful attention to their amino acid composition and sequence is necessary

(presence of residues containing amines, carboxylates and cysteines). For polysaccharides, the effects of oxidation on their overall structure must be considered. Synthetic design of the peptides and other haptens allows for the addition of unique functional groups that can be used for conjugation without affecting the intended epitopes.

An Example of the Mannich Reaction



Examples of Active Hydrogen Compounds That Can Participate in the Mannich Reaction

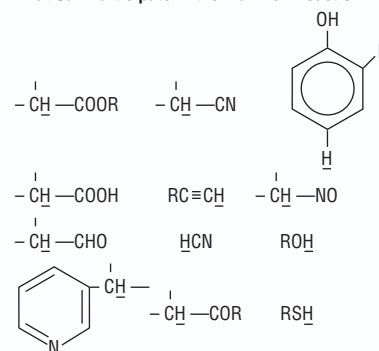


Figure 6. Examples of Mannich Reaction and active hydrogen compounds in Mannich Reaction.

PharmaLink™ Immunogen Kit

Conjugates haptens, such as drugs or steroids, that contain no easily reactive functional groups.

The PharmaLink™ Kit uses the Mannich reaction to conjugate the primary or secondary amines on a carrier protein to any available reactive hydrogens on the hapten (Figure 6). The figure shows examples of active hydrogen compounds that can participate in the Mannich reaction.

The use of SuperCarrier® Immune Modulator as a carrier protein generates a stronger antibody response than the use of BSA as a carrier.

Anti-hapten antibodies can be isolated without copurifying anti-carrier protein antibodies. By using the same conjugation chemistry to immobilize the hapten on a beaded support, a specific affinity column can be created to purify anti-hapten antibodies. If using the PharmaLink™ Immunogen Kit to generate antibodies, then use the Pierce PharmaLink™ Immobilization Kit (Product # 44930) to immobilize the hapten to a beaded support for antibody purification.

Highlights:

- Ten simple conjugations per kit
- Used to make immunogens against drug molecules or small metabolites
- Forms a stable secondary amine linkage by condensing formaldehyde between the hapten and the carrier protein functional groups

References

1. Muckerheide, A., et al. (1987). *J. Immunol.* **138**, 833-837.
2. Muckerheide, A., et al. (1987). *J. Immunol.* **138**, 2800-2804.
3. Domen, P.L., et al. (1987). *J. Immunol.* **139**, 3195-3198.
4. Apple, R.J., et al. (1988). *J. Immunol.* **140**, 3290-3295.
5. Harlow, E. and Lane, D. (1988). *Antibodies: A Laboratory Manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, pp. 56-100. (Product # 15050). *This manual discusses the use of carrier proteins in detail.*
6. Ranadive, N.S. and Sehon, A.H. (1967). *Can. J. Biochem.* **45**, 1701-1710.

Ordering Information

Product #	Description	Pkg. Size
77158	PharmaLink™ Immunogen Kit	Kit
	Includes: Imject® SuperCarrier® Immune Modulator	10 x 2 mg
	PharmaLink™ Conjugation Buffer	30 ml
	PharmaLink™ Coupling Reagent	4 ml
	Purification Buffer Salts	25 g
	Imject® Alum	50 ml
	Dextran Desalting Columns	10 x 5 ml



Assaying and Purifying Hapten-specific Antibodies

An important component in every antibody production procedure is the development of methods to assay immune serum for the hapten-specific antibody and then to purify it. If a carrier protein was used to prepare the immunogen, antibodies will be produced against both hapten and carrier. Therefore, assay or purification methods must be designed to discriminate between hapten-specific and carrier protein-specific antibodies.

To measure the specific anti-hapten response, perform the following immunoassay (ELISA): Couple the hapten to a non-relevant carrier protein by the same coupling chemistry (e.g., maleimide) used to prepare the immunogen. Coat this conjugate in an immunoassay microplate (e.g., Product # 15041) using overnight incubation in pH 9.4 carbonate-bicarbonate buffer (Product # 28382). Wash and block the plate wells with appropriate reagents. Add the test immune serum to test wells and normal (non-immune) control serum to other wells. Incubate the plate 1 hour to allow antibodies to bind. Wash the plate wells. Add an enzyme-conjugated detecting secondary antibody (e.g., Product # 31430 or 31460) and incubate the plate for 1 hour to allow binding to occur. Finally, wash the plate and detect active conjugated enzyme with an appropriate substrate (e.g., Product # 34028).

The dilution factor at which no anti-peptide antibody binding can be observed is called the antibody titer. One immunization protocol, for example, may produce an antibody titer of 1:10,000 dilution vs. a second protocol that may produce an antibody titer of only 1:5,000. The higher the dilution factor, the stronger the polyclonal immune response will be. Bovine serum albumin (BSA; 67,000 MW) and ovalbumin (OVA; 45,000 MW) are often used as non-relevant carrier proteins to assess anti-peptide antibody titers when mCKLH has been used as the immunogen.

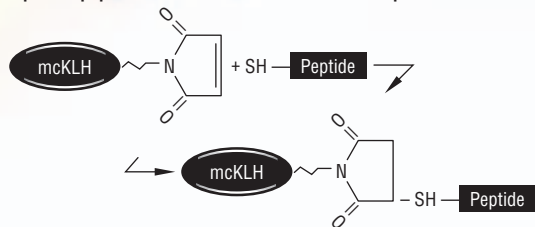
Purification of hapten-specific antibodies requires the same approach: immobilizing hapten to a solid support in a form that does not contain the same carrier protein used to prepare the immunogen. The *Affinity Purification Handbook* from Pierce describes in greater detail a number of activated affinity supports that can be used to immobilize haptens by many of the same chemical methods used for preparing hapten-carrier conjugates. EZ™ Antibody Production and Purification Kits (page 18) provide mCKLH carrier protein and activated affinity supports for both preparation of immunogen and purification of resulting antibodies.

EZ™ Antibody Production and Purification Kits

Everything you need to economically produce and purify antibodies ... just add the hapten!

Only Four Main Steps: An Example of Anti-Peptide Antibody Purification using Imject® Sulfhydryl Reactive Antibody Production and Purification Kit with mCKLH

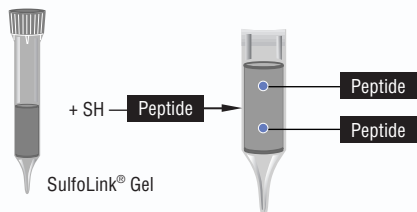
1. Couple the peptide to a maleimide-activated carrier protein.



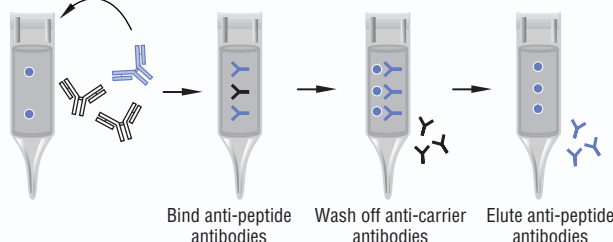
2. Immunize the animal to generate anti-peptide antibodies and anti-carrier antibodies.



3. Immobilize the peptide to SulfoLink® Gel in a column.



4. Purify anti-peptide antibodies away from anti-carrier antibodies.



For the ultimate convenience, use these kits to produce and purify your anti-peptide antibodies while removing anti-carrier antibodies. When you purchase one of these kits, you enjoy significant cost savings over ordering items individually.

Highlights:

- Available with your choice of conjugation chemistries: sulfhydryl-reactive for antigens with a terminal cysteine or carboxyl-reactive for antigens without a terminal cysteine
- These kits use the same reactive groups to conjugate your antigen to the carrier and the affinity support, allowing for proper orientation of the antigen during the antibody purification step
- Complete product instructions included

Reference

Hisamatsu, T., et al. (2003). *J. Biol. Chem.* **278**, 32962-32968.

Ordering Information

Product #	Description	Pkg. Size
77614	EZ™ Sulfhydryl Reactive Antibody Production and Purification Kit with mCKLH Includes: Imject® Maleimide Activated mCKLH Conjugation and Purification Buffers Gel Filtration Columns SulfoLink® Column Sample Preparation, Coupling and Wash Buffers β-Mercaptoethanol Cysteine	Kit 2 mg 1 x 5 ml 2 ml 6 mg 100 mg
77627	EZ™ Carboxyl Reactive Antibody Production and Purification Kit with mCKLH Includes: Imject® Maleimide Activated mCKLH Conjugation and Purification Buffers Gel Filtration Column Diaminodipropylamine Columns Coupling and Wash Buffers EDC	Kit 2 mg 1 x 5 ml 2 ml 50 mg

Determining Antibody Concentration

Concentration vs. Titer

Successful and reproducible antibody labeling and immunoassays are contingent on accurate information about the concentration and functional titer of purified antibodies. Concentration and titer are not equivalent. Concentration is the total amount of antibody without regard to its function. Depending upon the methods of purification employed, only a percentage of the total antibody concentration is composed of intact, active and functioning antibody with regard to its ability to bind antigen and yield a measurable response in an immunoassay. The titer of an antibody is the useful dilution of antibody in a given immunoassay. This is determined as the greatest dilution of an antibody preparation that

yields a response in that assay through a series of dilutions and is a functional measure of the activity of that antibody preparation. Some knowledge of both the concentration and titer is often helpful in optimizing the purification of an antibody and in subsequent use.

Antibody Concentration

The concentration of pure antibodies can be estimated from the absorbance measured at 280 nm, using an extinction coefficient of 13.5 for a 1% solution of IgG (10 mg IgG/ml). The concentration of pure antibodies can also be measured using a protein assay such as the BCA™ Protein Assay (Product # 23225) or Coomassie Plus – The Better Bradford™ Assay Kit (Product # 23236). Using

an immunoglobulin of known concentration (e.g., bovine gamma globulin, Product # 23212) as a standard, accurate determination of antibody concentration is possible with these protein assays.

Often, antibodies are not available in purified form and must be quantitated in serum, ascites fluid or culture supernatants. The increased use of antibodies as tools for research, diagnostic and therapeutic purposes has led to a demand for methods that can accurately determine antibody concentrations in these heterogeneous mixtures.

Pierce Easy-Titer® Assay Kits are simple, mix-and-read assays that allow the accurate determination of antibody concentrations from 15-300 ng/ml in about 30 minutes. The assay uses monodispersed polystyrene beads that bind to specific antibodies and absorb light at 340 and 405 nm. When the beads are mixed with a sample containing their target antibody, they aggregate and their ability to absorb light is decreased (Figure 7). Because this is an aggregation assay, low antibody concentrations yield high absorbance values, while high antibody concentrations yield low absorbance values. The decrease is proportional to antibody concentration and a standard curve can be generated to accurately quantitate levels of IgG in a variety of samples. Easy-Titer® Assay Kits feature a simple procedure that reduces hands-on time by using fewer steps and leads to more reproducible results. The antibody-binding beads are added to each well of a 96-well plate. Sample containing antibody is added to the wells, and this mixture is incubated for 5 minutes. Blocking reagent is added to the wells and the plate is incubated for another 5 minutes. The absorbance of each well at 340 or 405 nm is read on a 96-well plate reader. The entire process can be completed easily in about 30 minutes, unlike standard ELISA techniques that can require several hours.

Each Easy-Titer® Assay Kit is specific for a particular antibody species and isotype. For example, the Easy-Titer® Human IgG Assay Kit is specific for the human gamma chain and yields a uniform response with human IgG molecules of all subclasses (IgG₁, IgG₂, IgG₃ and IgG₄). It does not cross-react with other classes of human antibodies (IgM, IgA, IgD and IgE). In addition, the kit does not cross-react with antibodies from other species such as bovine antibodies present in the media used to culture antibody-producing hybridoma cells. This remarkable specificity allows the measurement of human IgG concentrations from a variety of sample types such as culture supernatants, ascites or body fluids without first purifying the antibody from other contaminants.

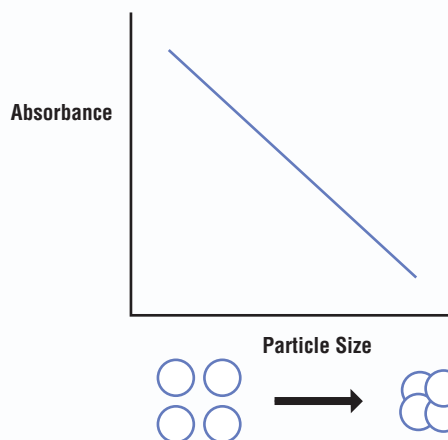


Figure 7. The assay principle behind the Easy-Titer® Human IgG Assay Kit.

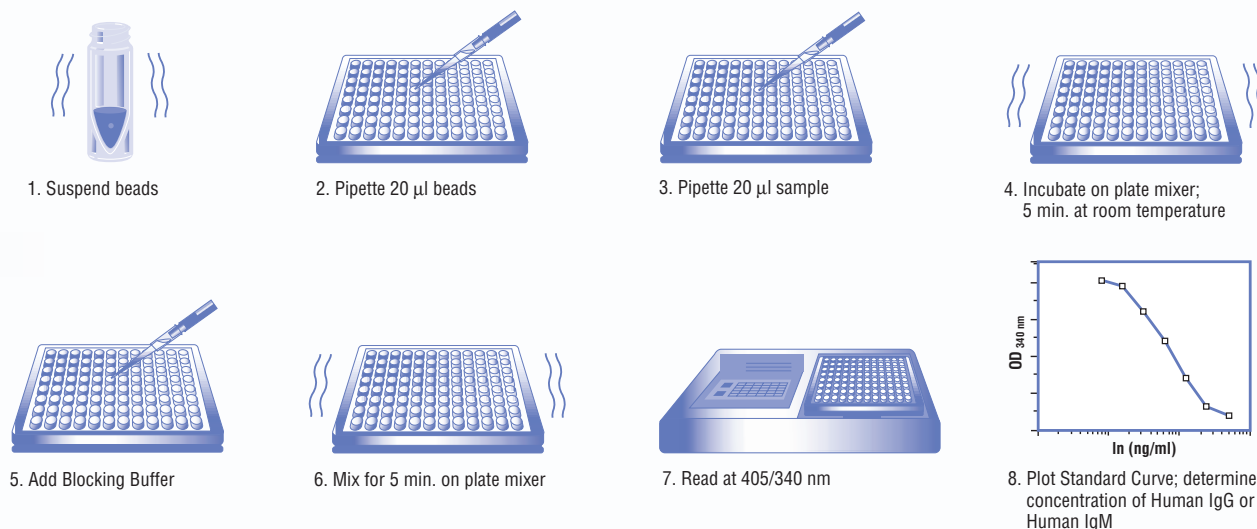


Figure 8. Protocol for the Easy-Titer® Human IgM Assay Kit.

Easy-Titer® IgG Assay Kits

The fastest, easiest way to quantitate antibodies ... ever!

It is no longer necessary to wait on or to rely on inaccurate and insensitive UV or colorimetric IgG determination methods. It is even possible to avoid the tedious time-consuming ELISA approach to determine antibody concentration. Easy-Titer® IgG Assay Kits make it possible to detect IgG in less time and with greater specificity and sensitivity than ever before.

Highlights:

- Fast – 10-minute total assay time
- Detection requires only measuring absorbance at 340 or 405 nm
- Sufficient for 96 assays – up to 87 determinations and a standard curve on a single plate
- Ready-to-use, three-component kit is easy to use
- Requires only a microplate, a shaker and a microplate reader
- Measures antibodies from culture supernatants, ascites or body fluids
- Measures humanized antibodies and chimeras with intact Fc regions

How the assay works:

- Monodisperse beads sensitized with a specific antibody absorb at 340 and 405 nm
- The beads agglutinate in the presence of human IgG or IgM
- Larger diameter clusters form that absorb less efficiently at 340 and 405 nm
- This decrease in absorbance is proportional to antibody concentration

Human IgG Assays

- Specificity
 - Specific for human IgG (all subclasses)
 - No cross-reactivity with human IgA, IgD, IgE or IgM or with IgG from other species
- Sensitivity
 - 15 ng/ml detection limit
 - 15-300 ng/ml detection range
- Coefficient of variation
 - <5% intra- and interassay

Human IgM Assay

- Specificity
 - Specific for human IgM
 - No cross-reactivity with human IgG or with IgM from other species
- Sensitivity
 - 15 ng/ml detection limit
 - 15-300 ng/ml detection range
- Coefficient of variation
 - <5% intra- and interassay

Easy-Titer® Assay Kits do not cross-react with antibodies from other species such as bovine antibodies present in the media used to culture antibody-producing hybridoma cells. This remarkable specificity allows the measurement of human IgG concentration from a variety of sample types such as culture supernatants, ascites or body fluids without first purifying the antibody from other contaminants.

Ordering Information

Product #	Description	Pkg. Size
23310	Easy-Titer® Human IgG (H+L) Assay Kit* <i>Sufficient material for 96 individual microplate assays, 88 assays and 7 point standard curve and blank.</i> Includes: Goat Anti-Human IgG Sensitized Polystyrene Beads [Monodisperse, polystyrene IgG (Fc) sensitized beads are supplied suspended in a phosphate buffer, pH 7.4 and stabilized with BSA and 0.1% azide] Easy-Titer® Dilution Buffer Easy-Titer® Blocking Buffer	Kit 2 ml 30 ml 15 ml
Human IgG Standard and microplates are available separately (see below).		
23325	Easy-Titer® Human IgG (gamma chain) Assay Kit* Includes: Goat Anti-Human IgG (γ chain) Sensitized Beads Easy-Titer® Dilution Buffer Easy-Titer® Blocking Buffer	Kit 2 ml 30 ml 15 ml
23315	Easy-Titer® Human IgM Assay Kit* Includes: Goat Anti-Human IgM Sensitized Polystyrene Beads Easy-Titer® Dilution Buffer Easy-Titer® Blocking Buffer	Kit 2 ml 30 ml 15 ml

Pierce offers the same easy-to-use assay technology for Mouse and Rabbit IgG quantization.

23300	Easy-Titer® Mouse IgG Assay Kit* Key Component: Goat Anti-Mouse IgG Sensitized Beads	Kit 2 ml
23305	Easy-Titer® Rabbit IgG Assay Kit* Key Component: Goat Anti-Rabbit IgG Sensitized Beads	Kit 2 ml

IgG Standards for Easy-Titer® Kits

31154	Human IgG, Whole Molecule	10 mg
31146	Human IgM, Whole Molecule	2 mg
31204	Mouse IgG, Whole Molecule	10 mg
31235	Rabbit IgG, Whole Molecule	10 mg

Microplates

15041	Reacti-Bind™ 96-Well Plates – Corner Notch	100 plates
15031	Reacti-Bind™ 8-Well Strip Plates – Corner Notch <i>Includes one strip well ejector per package.</i>	100 plates

* Easy-Titer® IgG Assay Technology is protected by U.S. patent # 5,043,289 and European patent # 0266278B1.

Isotyping Antibodies

Importance of Isotype Determination

Determining the class and subclass identity of an antibody is especially important for choosing by what method it should be purified and used in immunoassays. For example, if an antibody is determined to be IgM, it cannot be purified effectively with Protein A or G, and it will most likely require fragmentation for use in immunohistochemical procedures. If a monoclonal antibody is determined to be IgG₁ composed of kappa light chains, there is a good possibility that immobilized Protein L can be used to purify it from culture supernatant without contamination of bovine immunoglobulins from the serum supplement.

ImmunoPure® Monoclonal Antibody Isotyping Kits

ImmunoPure® Monoclonal Antibody Isotyping Kits use one of two colorimetric substrate detection systems and rabbit anti-mouse antibodies to determine mouse antibody isotype in an ELISA procedure. Kit I (Product # 37501) uses HRP/ABTS as the enzyme-substrate system and Kit II (Product # 37502) uses AP/PNPP.

Sufficient reagents and specific antibodies are supplied in each kit to perform one of two basic types of screening procedure. In the antigen-dependent method (Figure 9), antigen is coated in ELISA plates wells. The test hybridoma supernatant is then added to the wells and allowed to bind to the coated antigen. The antigen-bound monoclonal antibody is detected first with subclass-specific rabbit anti-mouse IgG (a different subclass-specific antibody in each well), then with an enzyme-conjugated anti-rabbit antibody. The antigen-independent screening method is used when soluble forms of the antigen are difficult to obtain. ELISA plate wells are coated with a general goat anti-mouse antibody, which serves to capture the test monoclonal antibody from the hybridoma supernatant. Finally, the captured monoclonal is detected with specific and secondary antibodies, as in the antigen-dependent method.

The kits identify antibodies as IgG, IgA or IgM; as IgG₁, IgG_{2a}, IgG_{2b} or IgG₃; and as having kappa or lambda light chains. The method requires that only one mouse antibody be present in the sample. It is not quantitative and will not effectively identify the predominant immunoglobulin subclasses in polyclonal serum or even in ascites.¹

Antigen-Dependent Monoclonal Antibody Isotyping

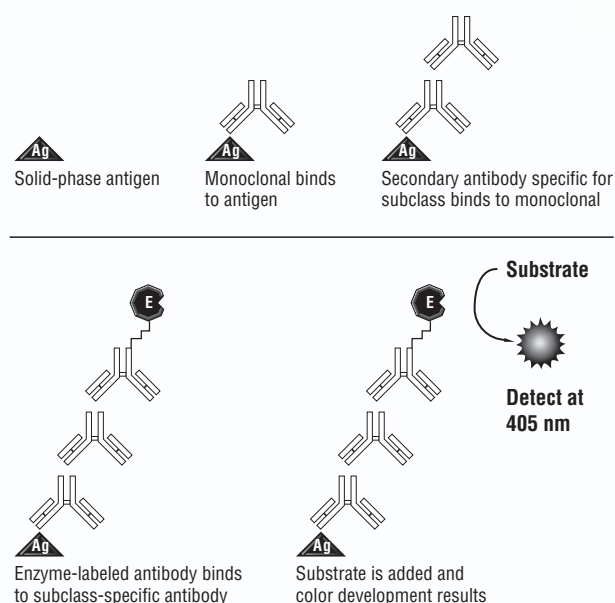


Figure 9. Antigen-dependent monoclonal antibody isotyping.

Reference

1. Reddy, K.B., *et al.* (2001). *J. Biol. Chem.* **276**, 28300-28308.

Monoclonal Antibody Isotyping Kits

Use the specificity of antibodies to determine the isotype of monoclonals.

The isotyping ELISA is a special type of ELISA in which the specificity of antibodies is used to determine the isotype of monoclonals. Pierce offers two ImmunoPure® Mouse Monoclonal Antibody Isotyping Kits: Horseradish Peroxidase (HRP)/ABTS and Alkaline Phosphatase (AP)/PNPP. There are two basic types of screening procedures for monoclonals. Antigen-dependent screening involves coating the antigen on a microplate. The hybridoma supernatant is then added, and the monoclonal is detected using an enzyme-conjugated antibody (see Figure 9, previous page). The antigen-independent screening method is used when soluble antigens are difficult to obtain. ELISA plates are coated with an antibody to mouse immunoglobulin. This antibody then serves to capture the monoclonal antibodies from the hybridoma supernatant. The presence of positive clones is proven with enzyme-conjugated anti-mouse immunoglobulin.

Highlights:

- Can be used for either antigen-dependent or antigen-independent procedures
- Results can be read either qualitatively using visual inspection or quantitatively with a microplate reader and a 405 nm filter

References

1. Liang, B., *et al.* (2000). *J. Immunol.* **165**, 3436-3443.
2. Kwon, B.S., *et al.* (1998). *FASEB J.* **12**, 845-854.
3. McKay, D.B., *et al.* (2003). *J. Bacteriol.* **185**, 2944-2951.

Ordering Information

Product #	Description	Pkg. Size
37501	ImmunoPure® Monoclonal Antibody Isotyping Kit I (HRP/ABTS) <i>Sufficient reagents to characterize 100 mouse monoclonal antibodies (1,000 wells).</i> Includes: Normal Rabbit Serum Rabbit Anti-Mouse IgG ₁ , IgG _{2a} , IgG _{2b} , IgG ₃ , IgA, IgM, kappa and lambda light chain Horseradish Peroxidase Conjugated Goat Anti-Rabbit IgG (H+L) 10X Substrate Buffer 50X ABTS Tween®-20 Positive Control (IgG ₁) Coating Antibody (Goat Anti-Mouse IgG + IgA + IgM) 50X Blocking Solution	Kit 6 ml 6 ml ea. 1.5 ml 10 ml 2 ml 2.5 ml 1 ml 2.5 ml 2.5 ml
37502	ImmunoPure® Monoclonal Antibody Isotyping Kit II (AP/PNPP) <i>Sufficient reagents to characterize 100 mouse monoclonal antibodies (1,000 wells).</i> Includes: Normal Rabbit Serum Rabbit Anti-Mouse IgG ₁ , IgG _{2a} , IgG _{2b} , IgG ₃ , IgA, IgM, kappa and lambda light chain Alkaline Phosphatase Conjugated Goat Anti-Rabbit IgG (H+L) 10X Substrate Buffer 50X PNPP Tween®-20 Positive Control (IgG ₁) Coating Antibody (Goat Anti-Mouse IgG + IgA + IgM) 50X Blocking Solution	Kit 6 ml 6 ml ea. 1.5 ml 10 ml 2 ml 2.5 ml 1 ml 2.5 ml 1 ml

Immunodiffusion Plates

Pre-cast Immunodiffusion Plates provide antibody-antigen precipitation detection.

Highlights:

- Gelling Agent contains precipitin brighteners, along with diffusion enhancers that help speed the interaction process
- Good precipitin bands from all species (including rabbit)
- Gels can be washed, dried and stained for a permanent record

Ordering Information

Product #	Description
31111	Immunodiffusion Plates, Agarose Gelling Agent 4 pattern/plate, 6 plates/pkg.
31113	Immunodiffusion Plates, Agarose Gelling Agent 1 pattern/plate, 10 plates/pkg.

Adjuvants

To enhance the immune response to an immunogen, various additives called adjuvants can be used. When mixed and injected with an immunogen, an adjuvant will enhance the immune response. An adjuvant is not a substitute for a carrier protein because it enhances the immune response to immunogens but cannot itself render haptens immunogenic. Adjuvants are nonspecific stimulators of the immune response, helping to deposit or sequester the injected material and causing a dramatic increase in the antibody response.

There are many popular adjuvants, including Freund's complete adjuvant (FCA). This reagent consists of a water-in-oil emulsion and killed *Mycobacterium*. The oil-and-water emulsion localizes the antigen for an extended period of time, and the *Mycobacterium* attracts macrophages and other appropriate cells to the injection site. Imject® Freund's Complete Adjuvant is used for the initial injections. Subsequent boosts use immunogen in an emulsion with Imject® Freund's Incomplete Adjuvant, which lacks the *Mycobacterium* component. Freund's adjuvants are very effective, but they do pose risks to both animal and researcher because of the toxic *Mycobacterium* components.

Imject® Alum is an equally effective and more convenient alternative to Freund's Adjuvants. Sample preparation with Imject® Alum involves simply mixing the antigen with a volume of adjuvant solution, followed by injection. SuperCarrier® Immune Modulator-conjugated immunogens, when used with Imject® Alum, yield an enhanced immune response – without the hassle or danger to animals associated with Freund's adjuvants.

Another alternative to FCA is AdjuPrime™ Immune Modulator. AdjuPrime™ Immune Modulator is a carbohydrate vaccine adjuvant that is nontoxic and easily administered in a single-phase aqueous suspension. These unique characteristics make it much easier and safer to use than FCA. AdjuPrime™ Immune Modulator is a macrophage-targeted, sustained antigen release adjuvant. It delivers a broad spectrum of macromolecular and oligopeptide antigens to the macrophage cell surface and stimulates the humoral response to the antigen. AdjuPrime™ Immune Modulator generates an antibody response almost equivalent to FCA but without the associated inflammatory response or granuloma formation.

Ordering Information

Product #	Description	Highlights	Disadvantages	Reference(s)	Pkg. Size
77140	Imject® Freund's Complete Adjuvant (FCA)	<ul style="list-style-type: none"> Enhances immune response to immunogen Used for initial injections Water-in-oil emulsion and killed <i>Mycobacterium</i> 	<ul style="list-style-type: none"> Difficult to mix with immunogen Can cause tissue necrosis at injection site 	1-3	5 x 10 ml
77145	Imject® Freund's Incomplete Adjuvant (FIA)	<ul style="list-style-type: none"> Used for subsequent boosts after initial injection No <i>Mycobacterium</i> present; mix with immunogen 	<ul style="list-style-type: none"> Difficult to mix with immunogen 	3	5 x 10 ml
77138	AdjuPrime™ Immune Modulator	<ul style="list-style-type: none"> Nontoxic, water-soluble, carbohydrate-based adjuvant Elicits about 70% of the immune response elicited by FCA Mixes easily with immunogen in aqueous solutions No harmful side effects Stimulation of IL-1 (T cell stimulation) 	<ul style="list-style-type: none"> Does not elicit as strong an immune response as FCA 	4-5	20 mg
77161	Imject® Alum	<ul style="list-style-type: none"> Unsurpassed convenience – ready for injection Specially made suspension of aluminum hydroxide and magnesium hydroxide allows you to mix your antigen with Imject® Alum Ideal for use with SuperCarrier® Immune Modulator 	<ul style="list-style-type: none"> Does not elicit as strong an immune response as FCA 	6-8	50 ml

References

- Heymach, Jr., J.V., *et al.* (1995). *J. Biol. Chem.* **270**(20), 12297-12304.
- Smith, J.W., *et al.* (1990). *J. Biol. Chem.* **265**(19), 11008-11013.
- Stanley, S., *et al.* (1995). *J. Biol. Chem.* **270**(28), 16694-16700.
- La Cava, A., *et al.* (2000). *J. Immunol.* **164**(3), 1340-1345.
- Russell, S.J., *et al.* (1996). *J. Biol. Chem.* **271**, 32810-32817.
- Iijima, N., *et al.* (2003). *Eur. J. Biochem.* **270**, 675-686.
- Morello, C.S., *et al.* (2002). *J. Virol.* **76**, 4822-4835.
- Rao, K.V.N., *et al.* (2003). *Clin. Diagn. Lab. Immunol.* **10**, 536-541.