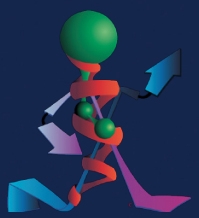
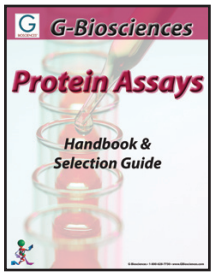
G-Biosciences

Antibody Production

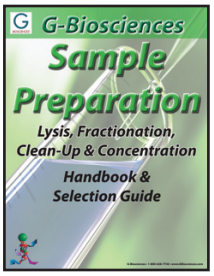
Handbook & Selection Guide

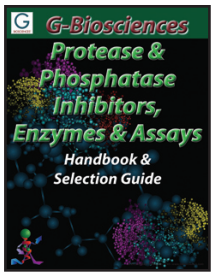




- Protein Estimation Assays
- Apoptosis Assays
- Cytotoxicity Assays
- SAM Methyltransferase Assays
- Protease Assays
- Phosphatase Assays
- Peroxide Assay

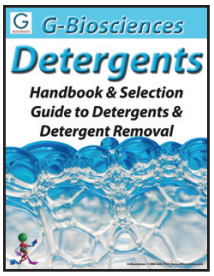
- Lysis Buffers & Systems
- Protein Fractionation Kits
- Dialysis (Micro) System
- Electrophoresis Clean-Up
- Concentration Systems
- Contamination Removal

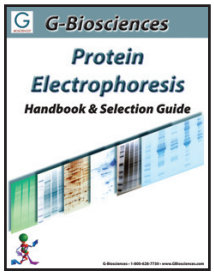




- Protease Inhibitor Cocktails
- Individual Protease Inhibitors
- Protease Assays
- Proteases for Mass Spec.
- Sequencing Grade Proteases

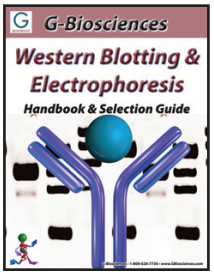
- Proteomic Grade Detergents
- Research Grade Detergents
- Non-Ionic, Ionic & Zwitterionic
- Detergent Estimations
- Detergent Removal Systems

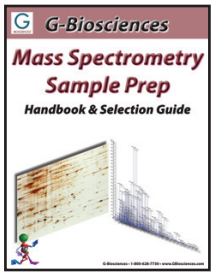




- Gel Preparation Chemicals
- Protein Marker Ladders
- Electrophoresis Buffers
- Reducing & Alkylating Reagents
- Protein Gel Stains

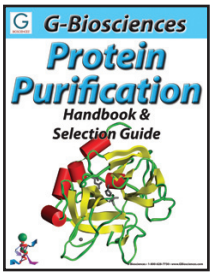
- 1-Hour Western System
- Transfer Buffers & Membranes
- Membrane Stains
- Blocking Buffers
- Secondary Antibodies
- Detection Reagents
- Reprobing Reagents

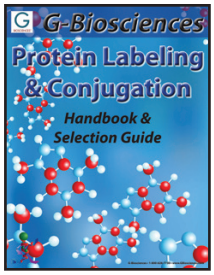




- Protein Sample Preparation
- Protein Clean-Up Systems
- Electrophoresis Reagents
- Mass Spec Grade Protease
- InGel Digestion Kits
- Peptide Generation Reagents

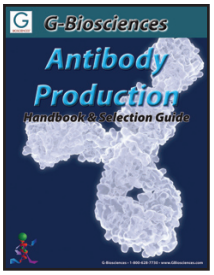
- Affinity Resins
- 6X His Protein Purification Kits
- GST Protein Purification Kits
- Antibody Purification
- Activated Resins
- Buffers & Reagents

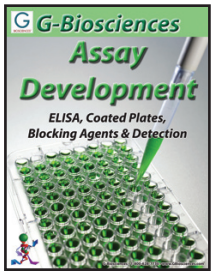




- Biotin Labeling
- Cell Surface Protein Labeling
- Agarose Coupling Kits
- Fluorescent Dye Labeling Kits
- Enzyme Labeling Systems

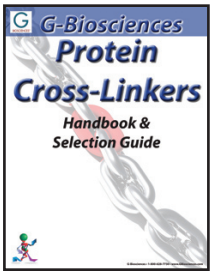
- Carrier Proteins
- Peptide Coupling Systems
- Antibody Purification Resins
- Antibody Fragmentation Kits

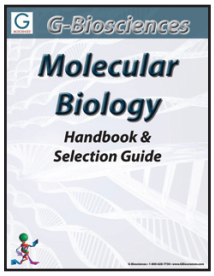




- Coated Plates
- Blocking Buffers
- Wash Buffers
- Secondary Antibodies
- Detection Reagents
- Antibody Labeling Systems

- Homobifunctional
- Heterobifunctional
- Optimizer Systems
- Cross-Linking Systems





- DNA Isolation
- Transformation & Screening
- Polymerase Chain Reaction
- Agarose Electrophoresis
- RNA Isolation
- Yeast Transformation

- Apoptosis Assays
- Cytotoxicity Assays
- SAM Methyltransferase Assays
- Protease Assays
- Phosphatase Assays
- Peroxide Assay
- ELISA

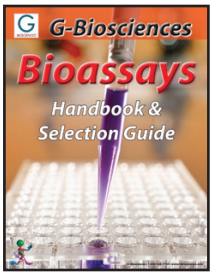


Table of Contents

Introduction

Antibodies, in particular the production of specific antibodies, have been essential in the advancement of many scientific fields, particularly proteomics, immunology and cell biology.

A common practice for the generation of specific antibodies is the use of immunogenic peptides derived from the protein of interest. These peptides are short sequences of amino acids that are antigenic, however they lack elements required for T cell activation. Activation is achieved with the use of “carrier proteins” that are coupled to the small antigenic peptide.

The Immune Response

The coupling of the peptide to a carrier protein is important in the stimulation of the immune response and the generation of antibodies. Below is a brief description and diagram summarizing the immune pathway responsible for detecting the peptide and carrier protein and generating subsequent antibodies.

The major histocompatibility complex (MHC) II pathway is responsible for the detection and generation of antibodies against foreign material, such as the carrier protein:peptide complex.

A B-cell or a circulating macrophage (antigen presenting cell (APC)) travels through the host’s blood stream in search of foreign material (carrier protein:peptide complex)(1). Once detected, the macrophage captures the carrier protein:peptide complex and brings it inside the cell, by a process known as endocytosis (2). The endosome, containing the carrier protein:peptide complex, moves towards the center of the cell and becomes acidified (3), at which time it may fuse with the lysosomes, which contain acid proteases. The acid proteases, in the acidified endosomes and lysosomes, digest the complex into small peptides (4).

The immature MHC class II (MHCII) proteins wait for the detection of foreign material in the endoplasmic reticulum, where it is in a complex with the Invariant chain (Ii) (5). Upon detection of the foreign material, the MHCII:Ii complex leaves the ER, traverses the Golgi and endosomes and finally fuses with the MHCII compartment (6). Cathepsins, in the MHCII compartment, partially digest Ii chain leaving CLIP in the peptide-binding site.

The endosome/lysosome, with the carrier protein:peptide peptides, fuses with the MHCII compartment and a human leukocyte antigen (HLA) protein replaces CLIP with a carrier protein:peptide peptide (7). The mature, peptide-loaded MHC class II protein moves to the plasma membrane and is presented on the cell surface (8).

A specific helper T-cell binds the peptide-loaded MHC class II protein through its T-cell receptor and CD4 receptor (9), which stimulates the macrophage to release interleukin-1 (IL-1). In turn, the helper T-cell releases IL-2 that stimulates itself and the macrophage (10). This stimulation and release of further interleukins and lymphokines leads to the proliferation of the macrophages and the helper T-cells, producing clones and some memory helper T-cells (important for fast secondary response). The released interleukins stimulate B lymphocyte cells (11) to initiate mitosis and cloning (12), producing more B lymphocyte cells and some memory B-cells (important for fast secondary response). After cloning, the B lymphocyte cells enlarge and develop an extensive endoplasmic reticulum and then begin mass-producing antibodies (13).

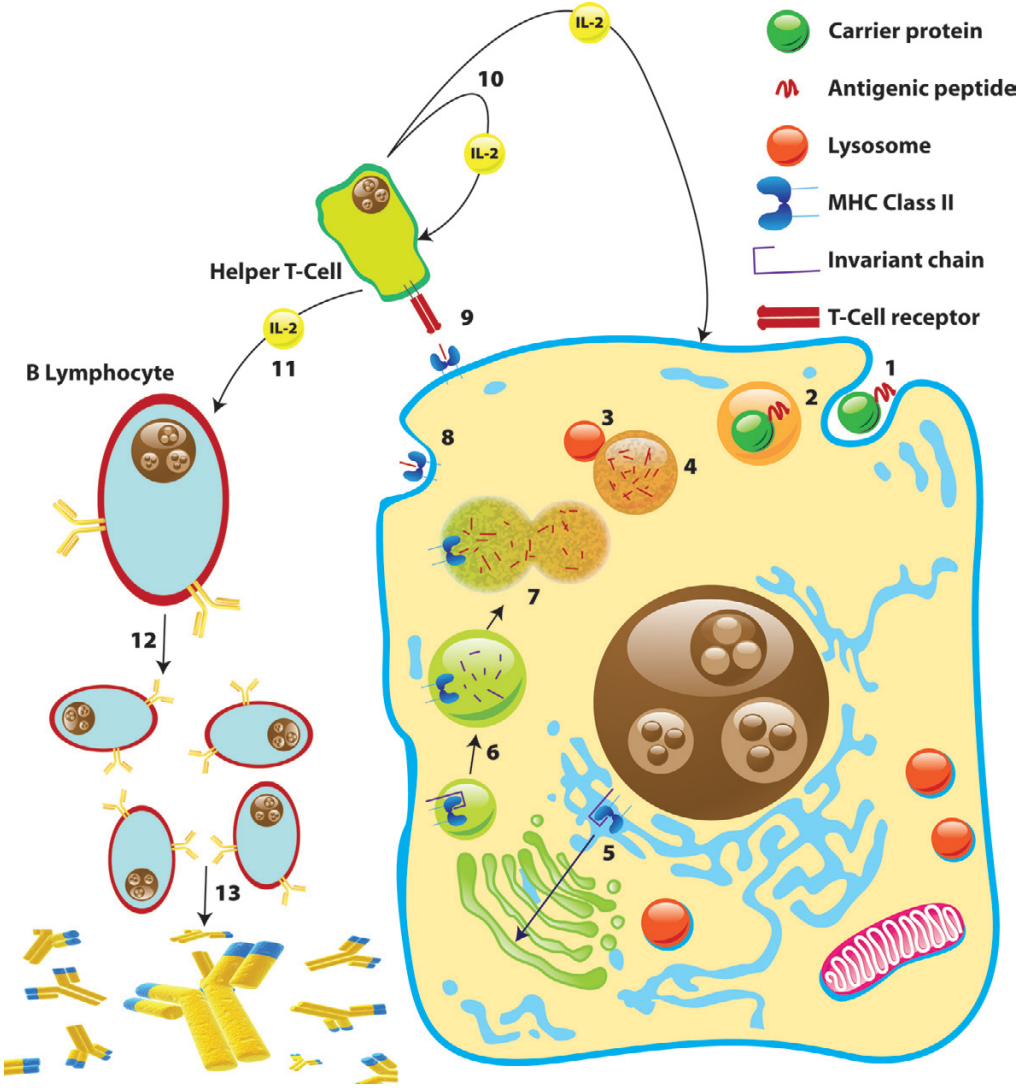


Figure 1: The Immune Response and Antibody production.

CARRIER PROTEINS

For the successful generation of antibodies

Many proteins are suitable for the role as a carrier protein and it is their properties that determine, to a large extent, the immune response and outcome of antibody production. Several factors are important to consider in the choice of the carrier protein. The first is the size of the carrier protein. Larger proteins (>60kDa) are preferable as it is highly probable that they contain the elements required for T-cell activation and they have multiple and sufficient numbers of exposed residues for peptide coupling, such as amine and sulfhydryl groups. An additional important factor in the choice of a carrier protein is to ensure that the carrier protein of choice is non-self, in fact the more genetically distinct the source the greater likelihood of a larger immunogenic response.

Keyhole Limpet Hemocyanin (KLH) is a commonly used carrier protein because it is purified from a mollusk (a gastropod) and is therefore very genetically distinct from the mammals used in antibody production. It is highly aggregated, giving it a molecular weight of $4.5x10^5$-$1.3x10^7$ kDa and has a large number of available lysine groups. Common problems with using KLH as a carrier protein are a result of its large degree of aggregation, which can lead to insolubility in aqueous solutions, and the large number of coupling sites which can lead to overloading of the antigenic peptide resulting in precipitation.

Bovine Serum Albumin (BSA) is another common carrier protein. It is smaller than KLH (67kDa), but still immunogenic. BSA is rich in lysine residues (59) of which 30-35 are available for coupling, it is highly soluble and stable making its preparation and use very simple.

Over the years, immunological researchers have focused their attention on trying to understand the antigen recognition pathways and subsequent immunogenic responses, including antibody production. Researchers were able to demonstrate that a cationized form of BSA, produced by replacing anionic side chain carboxylic groups with aminoethylamide groups, was more immunogenic than normal BSA[1]. They have shown that the amount of cationized BSA (cBSA) required for stimulation of T-cell proliferation in-vitro was 500 times less than normal BSA (nBSA), whereas in-vivo cBSA produced responses which were at least twice nBSA and lasted for longer periods of time. In addition, antibodies were produced in response to cBSA, in the absence of adjuvants, which was not the case for nBSA.

Further research demonstrated that cBSA exhibited unique immunogenic properties as a result of alterations in the self-regulation of the immune response[2]. Pretreatment with cBSA, either orally[3] or intravenously, prior to immunization with cBSA greatly enhanced the anti-BSA response; nBSA pretreatment suppressed this response.

The underlying mechanisms to the increased strength and duration of antibody responses are not fully understood. Researchers, however, believe that the increased positive charge (pI>10.5) of cBSA gives it greater affinity for the negative membrane surface of antigen presenting cells (APCs)[4] and have shown that cBSA is taken into the cell by an adsorptive mechanism, such as receptor mediated endocytosis, as opposed to the slower fluid phase pinocytosis utilized by nBSA[5]. This results in a more rapid and efficient uptake and subsequent processing of the antigen.

Interestingly, the enhanced immune response of cBSA can be extended to peptides and proteins coupled to cBSA, allowing for a greater immune response and therefore higher titer antibody production. HyperCarrier™ is a cationized BSA

REFERENCES

1. *Muckerheide, A., et al (1987) J. Immunol. 138: 833*
2. *Muckerheide, A., et al (1987) J. Immunol. 138: 2800*
3. *Domen, P.L., et al (1987) J. Immunol. 139: 3195*
4. *Dohlman, J.G.,et al (1991) Biochem. Biophys. Res. Commun. 181: 787*
5. *Apple, R.J.,et al. (1988) J. Immunol. 140: 3290*

KLH

Keyhole Limpet Hemocyanin

Keyhole limpet hemocyanin is a widely used carrier protein due to its large molecular mass. It is $4.5x10^6$-$1.3x10^7$Da aggregates composed of 350-390kDa subunits. The large size results in a large number of primary amines that can react with cross-linkers for the coupling of peptides. Supplied as KLH or ActiveHOOK™ KLH, a maleimide activated carrier protein, as 8 x 2mg single use OneQuant™ vials or 10mg vials.

FEATURES

- High molecular mass ($4.5x10^5$-$1.3x10^7$Da aggregates)
- Stronger immune response compared to BSA
- Convenient 8 x 2mg single use OneQuant™ vials or 10mg vials

APPLICATIONS

- Carrier protein for peptides for the production of antibodies

Cat. No.	Description	Size
786-088	KLH (Immunological Grade)	10mg
786-091	*OneQuant*™ KLH (Immunological Grade)	8 x 2mg
786-089	*Active*HOOK™ KLH	10mg
786-094	*OneQuant*™ *Active*HOOK™ KLH	8 x 2mg

Bovine Serum Albumin (BSA)

BSA is a single polypeptide of 67kDa and consists of 59 lysine residues, of which 30-35 have primary amines that can react with crosslinkers for the coupling of peptides. Supplied as BSA or ActiveHOOK™ BSA, a maleimide activated carrier protein, as 8 x 2mg single use OneQuant™ vials or 10mg vials.

FEATURES

- 59 lysine residues; 30-35 are capable of conjugating
- Single polypeptide protein (67kDa)
- More soluble, but less immunogenic, than KLH
- Convenient 8 x 2mg single use OneQuant™ vials or 10mg vials

APPLICATIONS

- Carrier protein for peptides for the production of antibodies

Cat. No.	Description	Size
786-086	Bovine Serum Albumin (BSA) (Immunological Grade)	10mg
786-090	*OneQuant*™ BSA (Immunological Grade)	8 x 2mg
786-087	*Active*HOOK™ BSA	10mg
786-093	*OneQuant*™ *Active*HOOK™ BSA	8 x 2mg

HyperCarrier™

A cationized BSA for a greater immune response

HyperCarrier™ is normal bovine serum albumin (BSA) that has been treated with ethylene diamine, which substitutes anionic carboxyl groups with cationic aminoethyl-amide groups.

Researchers demonstrated that cationized BSA was more immunogenic than normal BSA (1). They have shown that the amount of cationized BSA (cBSA) required for stimulation of T-cell proliferation in-vitro was 500 times less than normal BSA (nBSA), whereas in-vivo cBSA produced responses which were at least twice nBSA and lasted for longer periods of time. In addition, antibodies were produced in response to cBSA, in the absence of adjuvants, which was not the case for nBSA. Further research demonstrated that cBSA exhibited unique immunogenic properties as a result of alterations in the self-regulation of the immune response (2). Pretreatment with cBSA, either orally (3) or intravenously, prior to immunization with cBSA greatly enhanced the anti-BSA response; nBSA pretreatment suppressed this immune response.

Supplied as HyperCarrier™ or ActiveHOOK™ HyperCarrier™, a maleimide activated carrier protein, as 8 x 2mg single use OneQuant™ vials or 10mg vials.

FEATURES

- Increased binding to immune system cells
- Stronger immune response compared to BSA
- Single polypeptide protein (67kDa)
- Convenient 8 x 2mg single use OneQuant™ vials or 10mg vials

APPLICATIONS

- Carrier protein for peptides for the production of antibodies

REFERENCES

1. *Muckerheide, A., et al (1987) J. Immunol. 138: 833*
2. *Muckerheide, A., et al (1987) J. Immunol. 138: 2800*
3. *Domen, P.L., et al (1987) J. Immunol. 139: 3195*

Cat. No.	Description	Size
786-096	HyperCarrier™ (Immunological Grade)	10mg
786-092	*OneQuant*™ HyperCarrier™ (Immunological Grade)	8 x 2mg
786-097	*ActiveHOOK*™ HyperCarrier™	10mg
786-095	*OneQuant*™ *Active*HOOK™ HyperCarrier™	8 x 2mg

ACTIVATED CARRIER PROTEINS

ActiveHOOK™ Carrier Proteins

Malemide activated carrier proteins

The ActiveHOOK™ line of carrier proteins are maleimide activated by the addition of sulfoSMCC cross-linker. The ActiveHOOK™ line rapidly couples to free sulfhydryl groups on peptides and proteins. These activated carrier proteins save both time and money as separate cross-linkers are not required and the reaction is a one step reaction.

See individual carrier protein listings for more information.

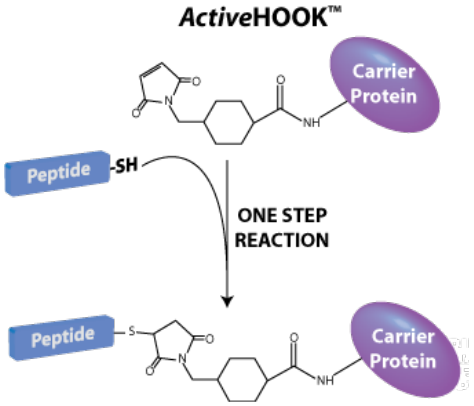


Figure 2: The coupling scheme for ActiveHOOK™ Carrier proteins.

Cat. No.	Description	Size
786-087	*Active*HOOK™ BSA	10mg
786-093	*OneQuant*™ *Active*HOOK™ BSA	8 x 2mg
786-097	*ActiveHOOK*™ HyperCarrier™	10mg
786-095	*OneQuant*™ *Active*HOOK™ HyperCarrier™	8 x 2mg
786-089	*Active*HOOK™ KLH	10mg
786-094	*OneQuant*™ *Active*HOOK™ KLH	8 x 2mg

PEPTIDE COUPLING KITS

HOOK™ Peptide Coupling (Amine Reactive)

Designed for the coupling of peptides to carrier proteins, utilizing the primary amines and carboxyl groups of the peptides and carrier proteins. This kit utilizes the chemical heterobifunctional crosslinker EDC to couple peptides to carrier proteins. EDC first reacts with the carboxyl groups, producing an amine reactive intermediate, O-acylisourea that rapidly reacts with the amine groups of the peptide/protein.

This kit utilizes Tube-O-Reactor™, which allows for the reactions to be performed in a single tube, with no loss of essential reagents and minimum hands on time and effort.

FEATURES

- Simple to use, single step reaction
- Suitable for all carrier proteins

APPLICATIONS

- Coupling of peptides to carrier proteins for antibody production

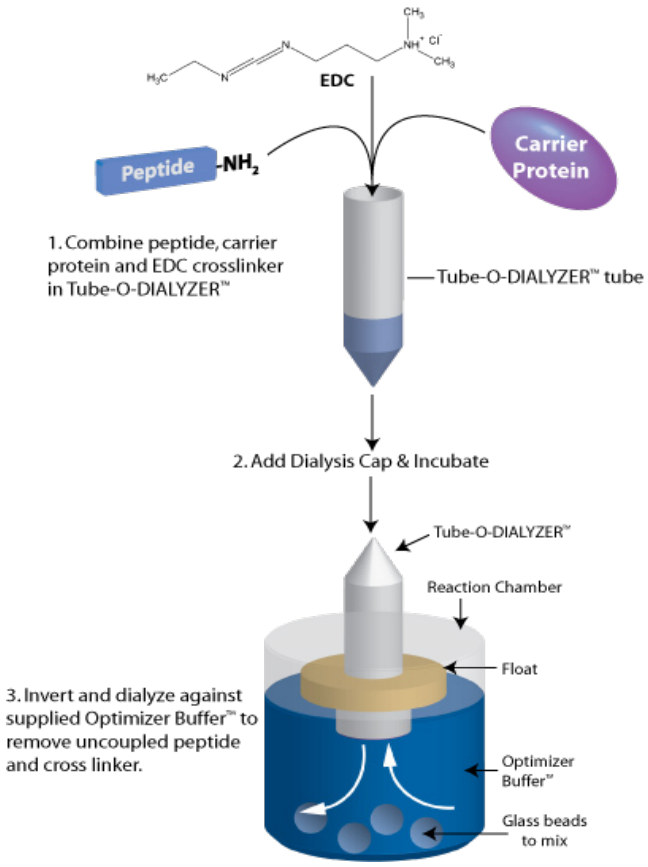


Figure 3: HOOK™ Peptide Coupling (Amine Reactive) scheme.

Cat. No.	Description	Size
786-067	HOOK™ Peptide Coupling Kit (Amine reactive)	5 Reactions
786-068	HOOK™ Peptide Coupling Kit (Amine reactive) with *OneQuant*™ BSA	5 Reactions
786-069	HOOK™ Peptide Coupling Kit (Amine reactive) with *OneQuant*™ KLH	5 Reactions
786-070	HOOK™ Peptide Coupling Kit (Amine reactive) with *OneQuant*™ HyperCarrier™	5 Reactions

HOOK™ Peptide Coupling (Sulfhydryl Reactive)

This kit is designed for the coupling of peptides to carrier proteins, utilizing a sulfhydryl group in the peptide.

This kit exploits the chemical cross-linker sulfoSMCC to couple peptides through their sulfhydryl groups, found on cysteine side chains, to the primary amines on the carrier proteins.

The N-hydroxysuccinimide (NHS) ester in sulfoSMCC reacts with primary amines to form covalent amide bonds and the maleimide group reacts with sulfhydryl groups to form stable thioether bonds.

If peptides do not contain a sulfhydryl group then G-Biosciences recommends and supplies Traut's reagent (2-IminothiolaneHCl). Traut's reagent modifies primary amines, located at the N-terminus and on lysine side chains, introducing a sulfhydryl group that is fully compatible with this kit.

This kit utilizes Tube-O-Reactor™, which allows for the reactions to be performed in a single tube, with no loss of essential reagents and minimum hands on time and effort.

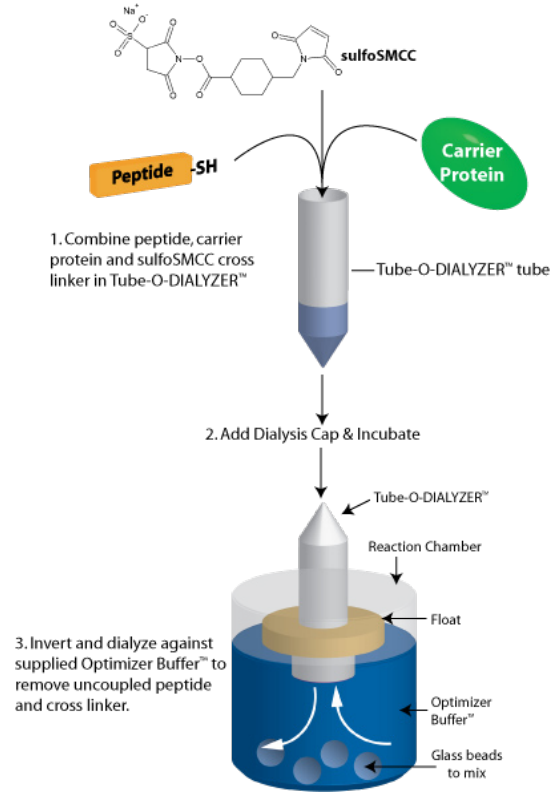


Figure 4: HOOK™ Peptide Coupling (Sulfhydryl Reactive) scheme.

FEATURES

- Simple to use
- Suitable for all carrier proteins
- Uses Tube-O-Reactor™ for minimum hands on time

APPLICATIONS

- Coupling of peptides to carrier proteins.
- Suitable for antibody production.

Cat. No.	Description	Size
786-071	HOOK™ Peptide Coupling Kit (Sulfhydryl reactive)	5 Reactions
786-072	HOOK™ Peptide Coupling Kit (Sulfhydryl reactive) with *OneQuant*™ BSA	5 Reactions
786-073	HOOK™ Peptide Coupling Kit (Sulfhydryl reactive) with *OneQuant*™ KLH	5 Reactions
786-074	HOOK™ Peptide Coupling Kit (Sulfhydryl reactive) with *OneQuant*™ HyperCarrier™	5 Reactions

PROTEIN A & PROTEIN G

Immobilized Protein A

For binding the constant domains of immunoglobulin (Ig) molecules (Table 19). Protein A is coupled to agarose beads by a proprietary coupling method that provides high coupling efficiency for immunoglobulins and minimal protein A leaching. Immobilized Protein A Resin is available as resin alone or supplied in a kit format containing:

- 5ml resin
- 100ml Protein A or G Binding/Wash Buffer *(0.1M sodium phosphate, 0.15M NaCl, pH7.5)*
- 100ml Protein A or G Elution Buffer *(100mM glycine, pH3.0)*
- 5 empty 1ml spin columns
- 5 empty <5ml gravity flow columns

The buffers are also available separately.

FEATURES

- For the binding of immunoglobulins
- High binding capacity: 18-43mg/ml resin
- Bead Structure: 6% highly cross-linked agarose

APPLICATIONS

- For immunoaffinity chromatography & immunoprecipitation

CITED REFERENCES

Shi, L. et al (2009) J. Biol. Chem. 284: 3966 - 3975

Cat. No.	Description	Size
786-283	Immobilized Protein A Resin	5ml resin
786-553	Immobilized Protein A Resin Kit	1
786-544	Protein A or G Binding/ Wash Buffer	100ml
786-545	Protein A or G Elution buffer	100ml

Immobilized Protein G

For binding the constant domains of immunoglobulin (Ig) molecules (Table 19). Protein G is a modified form of Streptococcal group G so that it does not bind to albumin. Protein G is coupled to 4% cross-linked agarose beads by a proprietary coupling method that provides high coupling efficiency for Ig and minimal protein G leaching. Immobilized Protein G Resin is available as resin alone or supplied in a kit format containing:

- 5ml resin
- 100ml Protein A or G Binding/Wash Buffer *(0.1M sodium phosphate, 0.15M NaCl, pH7.5)*
- 100ml Protein A or G Elution Buffer *(100mM glycine, pH3.0)*
- 5 empty 1ml spin columns
- 5 empty <5ml gravity flow columns

The buffers are also available separately.

FEATURES

- For the binding of immunoglobulins
- High binding capacity: >20mg Human IgG/ml resin
- Ligand density: ~2mg protein G /ml resin
- Bead size: 50-160µm
- Bead Structure: 4% highly cross-linked agarose

APPLICATIONS

- For immunoaffinity chromatography & immunoprecipitation

Cat. No.	Description	Size
786-284	Immobilized Protein G Resin	5ml resin
786-554	Immobilized Protein G Resin Kit	1
786-544	Protein A or G Binding/ Wash Buffer	100ml
786-545	Protein A or G Elution buffer	100ml

Species	Antibody Class	Protein A	Protein G	Protein A/G
Mouse	Total IgG	*****	*****	*****
	IgM	-	-	-
	IgG_1	*	***	***
	IgG_{2a}	*****	*****	*****
	IgG_{2b}	*****	*****	*****
	IgG_3	*****	*****	*****
Human	Total IgG	*****	*****	*****
	IgG_1	*****	*****	*****
	IgG_2	*****	*****	*****
	IgG_3	*	*****	*****
	IgG_4	*****	*****	*****
	IgM	*	-	*
	IgD	-	-	-
	IgA	*	-	*
	Fab	*	*	*
	ScFv	*	-	*
Rat	Total IgG	*	***	***
	IgG_1	*	***	***
	IgG_{2a}	-	*****	*****
	IgG_{2b}	-	*	*
	IgG_{2c}	*****	*****	*****
Rabbit	Total IgG	*****	*****	*****
Goat	Total IgG	*	*****	*****
	IgG_1	*	*****	*****
	IgG_2	*****	*****	*****
Cat	Total IgG	*****	*	*****
Chicken	Total IgY	-	-	-
Cow	Total IgG	*	*****	*****
	IgG_1	*	*****	*****
	IgG_2	*****	*****	*****
Dog	Total IgG	*****	*	*****
Guinea Pig	Total IgG	*****	*	*****
Horse	Total IgG	*	*****	*****
	IgG(ab)	*	-	*
	IgG(c)	*	-	*
	IgG(T)	-	*****	*****
Pig	Total IgG	*****	*	*****
Sheep	Total IgG	*	*****	*****
	IgG_1	*	*****	*****
	IgG_2	*****	*****	*****

Table 1: Relative affinity of Protein A, Protein G and Protein A/G for immunoglobulins.

PEARL™ PURIFICATION

Pearl™ IgG Purification Resin

For the one-step purifiication of the immunoglobulin G (IgG) antibodies from serum. The resin binds the high abundant, non-IgG proteins (i.e albumin) and allows the IgG molecules to pass through in a physiological buffer. The purified IgG molecules can be stored or used in further downstream applications without further clean-up, such as ammonium sulfate precipitation.

Purifies IgG in <15 minutes, which is more rapid than the commonly used Protein A and Protein G resins. The performance of the Pearl™ IgG Purification Resin is comparable or better than the Protein A and Protein G resins (Table 18).

Species	Pearl™ IgG Purification Resin	Protein A	Protein G
Mouse	++++	++++	++++
Human	++++	++++	++++
Rat	++++	+	++
Hamster	++++	++	++
Guinea Pig	++++	++++	++
Rabbit	++++	++++	+++
Horse	++++	++	++++
Cow	++	++	++++
Pig	++++	+++	++
Sheep	++	+	++
Goat	++++	+	++
Chicken	-	-	-

Table 2: Binding efficiencies of Pearl™ IgG Purification Resin compared to Protein A and Protein G.

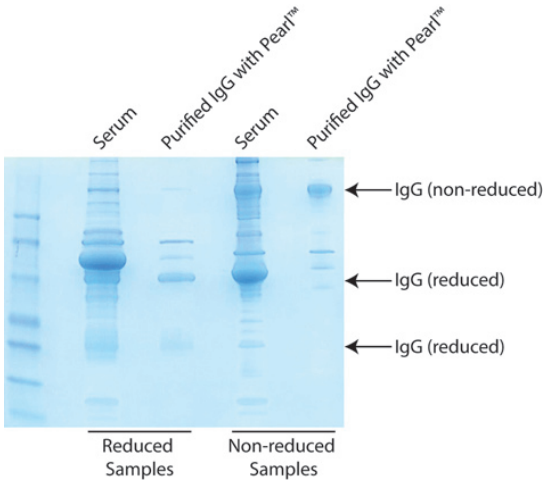


Figure 5: Pearl™ IgG Purification Resin rapidly purifies IgG molecules. Rabbit serum was dialyzed for 2 hours against IgG Purification Buffer and treated with IgG Purification Resin. The serum and flowthrough were compared under reducing and non reducing conditions.

FEATURES

- Simple 1-step purification
- High recovery (>90%) & Purity (>80%)

APPLICATIONS

- Purification of IgG (Immunoglobulin G) molecules
- Purify IgG from sources not compatible with Protein A & G

Cat. No.	Description	Size
786-800	Pearl™ IgG Purification Resin	3ml resin
786-801	Pearl™ IgG Purification Resin	25ml resin

Pearl™ IgG Purification

Isolate IgG in physiological buffer in <15mins

The Pearl™ IgG Purification kits are designed for the one step purification of IgG (Immunoglobulin G) antibodies from serum. The supplied Pearl™ IgG Purification Resin binds the high abundant, non-IgG proteins (i.e albumin) and allows the IgG molecules to pass through in a physiological buffer. The purified IgG molecules can be stored or used in further downstream applications without further clean-up, such as ammonium sulfate precipitation.

The Pearl™ IgG purification kits can purify IgG in <15 minutes, which is more rapid than the commonly used Protein A and Protein G resins. The performance of the Pearl™ IgG Purification Resin is comparable or better than the Protein A and Protein G resins (Table 18).

Pearl™ IgG Purification (Spin Format) kit is ideal for the rapid, small scale purification of IgG. The kit is supplied with 3ml Pearl™ IgG Purification Resin, IgG Isolation Buffer and 20 spin columns. Suitable for purifying up to 25mg IgG.

Pearl™ IgG Purification kit is supplied with 25ml Pearl™ IgG Purification Resin and IgG Isolation Buffer and is suitable for the isolation of IgG from ~100ml serum (~200mg IgG).

Cat. No.	Description	Size
786-798	Pearl™ IgG Purification (Spin Format) Kit	For 25mg IgG
786-799	Pearl™ IgG Purification Kit	For ~200mg IgG

Pearl™ Monoclonal IgG Purification

Isolate monoclonal antibodies from ascites & cell culture supernatant

The Pearl™ Monoclonal IgG Purification kit allows for the rapid purification of antibodies from cell culture supernatant and ascites fluid. The Pearl™ IgG Purification Resin binds the high abundant, non-IgG proteins (i.e. albumin) and allows the IgG molecules to pass through in a physiological buffer. The IgG molecules can be stored or used in downstream applications without further clean-up, such as ammonium sulfate precipitation.

The Pearl™ Monoclonal IgG Purification kit can be used to purify antibodies direct from cell culture supernatant with less than 10% FBS or can be used with ascites fluid after treatment with the supplied Ascites PreTreat.

The Pearl™ Monoclonal IgG Purification kit can purify IgG from ~1L cell culture supernatant or 200ml ascites fluid.

FEATURES

- Isolate monoclonal antibodies for ascites fluid or cell culture supernatant
- Supplied with ascites pretreatment reagent for optimal IgG purification
- For 1L of cell culture supernatant or 0.2L ascites fluid

APPLICATIONS

- Monoclonal antibody isolation from ascites fluid or cell culture supernatant

Cat. No.	Description	Size
786-802	Pearl™ Monoclonal IgG Purification Kit	1 kit

Pearl™ Antibody Clean Up Kit

Removal of inhibitory BSA & gelatin from antibody solutons

Purified and commercial antibodies are routinely stored in buffers containing bovine serum albumin (BSA) and gelatin that act as stabilizers during long term storage. In routine applications, such as ELISA, Western blotting and other immunodetection techniques, these proteins generally do not interfere. The presence of the protein stabilizers do interfere with antibody labeling and conjugation techniques, including biotinylation, fluorescent dye labeling, covalent antibody immobilization and antibody fragmentation experiments.

The Antibody Clean Up kit is designed for the rapid clean up of antibody solutions using a combination of our Pearl™ IgG Purification Resin to remove the protein stabilizers and SpinOUT™ desalting columns to ensure the antibody solutions are in an optimal buffer for clean up. The Pearl™ IgG Purification Resin binds the high abundant, non-IgG proteins (i.e. BSA and gelatin) and allows the IgG molecules to pass through in a physiological buffer.

For the purification of ten 0.5ml IgG samples with up to 1% BSA and gelatin.

FEATURES

- Remove BSA and Gelatin protein stabilizers
- SpinOUT™ columns to ensure optimal conditons for antibody clean up
- Pearl™ IgG Purification Resin for antibody clean up
- Suitable for 10 x 0.5ml IgG Samples

APPLICATIONS

- Remove BSA & Gelatin protein stabilizers that interfere with antibody labeling, fragmentation and isotyping experiments

Cat. No.	Description	Size
786-803	Pearl™ Antibody Clean Up	10 x 0.5ml samples

IgA PURIFICATION

Immobilized Jacalin

Jacalin, or *Artocarpus integrifolia* lectin, is a tetrameric two-chain lectin with a molecular weight of 66kDa. Jacalin is a α-D-galactose binding lectin purified from jack-fruit (*Artocarpus integrifolia*) seeds. Applications include isolating IgA from human serum and colostrums, isolating human plasma glycoproteins and histochemistry. Jacalin also binds IgD.

FEATURES

- Binding Capacity: 1-3mg human IgA/ml resin
- Loading: ≈4.5mg jacalin/ml of resin
- Support: 6% cross-linked agarose

APPLICATIONS

- Preparing Human IgA free of contaminating IgG

Cat. No.	Description	Size
786-167	Immobilized Jacalin	2ml resin

Jacalin, Lyophilized

Artocarpus integrifolia lectin

Jacalin, or *Artocarpus integrifolia* lectin, is also available as a lyophilized protein.

Cat. No.	Description	Size
786-473	Jacalin, lyophilized	10mg

THIOPHILIC ADSORPTION

Thiophilic Resin

For thiophilic adsorption of IgG, IgM, IgY and protein purification

Thiophilic adsorption or thiophilic chromatography is a routinely used technique for the low cost, simple purification of immunoglobulins. Thiophilic adsorption was first developed by Porath et al in 1984 and is a group specific, salt-dependent purification technique that has distinct affinity towards immunoglobulins and α_2-macroglobulins. The thiophilic adsorption works on the principle that some proteins in high salt are able to bind to an immobilized ligand that contains a sulfone group in proximity to a thioether group. The bound proteins are then eluted in decreasing salt concentrations.

The thiophilic resin binds immunoglobulins, including IgG, IgY and IgM, from serum, ascites or tissue culture supernatants and the purified immunoglobulins are then eluted in a near neutral aqueous buffer. The thiophilic resin has a high binding capacity (~20mg/ml human IgG/ml resin) and a broad specificity for various species' immunoglobulin molecules.

Thiophilic adsorption has been used to purify other proteins including horseradish peroxidase[2], glutathione peroxidase[3], lactate dehydrogenase[4] and allergens[5].

Supplied with protocols for IgG purification, IgM purification, IgY purification and general protein purification.

The Thiophilic Adsorption kit is supplied with the thiophilic resin and all the necessary buffers for the rapid purification of immunoglobulin G (IgG) antibodies.

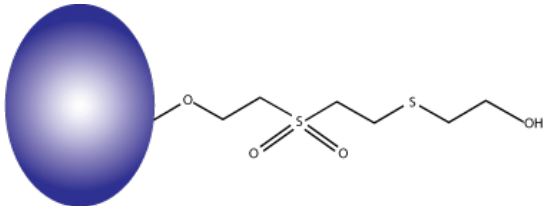


Figure 6: Structure of thiophilic group on agarose beads.

FEATURES

- Purify wide range of immunoglobulin molecules, including IgG, IgM and IgY
- High binding capacity (20mg human IgG/ml resin)
- Binds chicken immunoglobulin (IgY)
- Gentle elution conditions in very low salt and near neautral pH
- Adaptable to other proteins
- Enrichment alternative to ammonium sulfate precipitation

APPLICATIONS

- Purify immunoglobulins, including IgG, IgM and chicken IgY

REFERENCES

1. *Porath, J. et al (1984) In Physical Chemistry of Colloids and Macromolecules, Ed. Ranby, B. (Upsala, Sweden), p. 137*
2. *Chaga, G. et al (1992) Biomed. Chromatogr. 6:172*
3. *Huang, K. et al (1994) Biol. Trace Elem. Res. 46:91*
4. *Kminkova, M. & Kucera, J. (1998) Prep. Biochem. Biotechnol. 28:313*
5. *Goubran-Bostros, H. et al (1998) J. Chromatogr. B. Biomed. Sci. Appl. 710:57*

Cat. No.	Description	Size
786-266	Thiophilic Adsorption Kit	1 Kit
786-267	Thiophilic Resin	10ml resin
786-268	Thiophilic Resin	100ml resin

AFFINITY COLUMN GENERATION

Sulfhydryl Coupling Resin

Activated iodoacetyl group for binding free sulfhydryls

The Sulfhydryl Coupling Resin is designed for the simple and efficient coupling of peptides and proteins to a solid 6% agarose support through free sulfhydryl groups (-SH). The iodoacetyl groups of the Sulfhydryl Coupling Resin specifically react with free sulfhydryls to form covalent, permanent thioether bonds (see figure). The long spacer arm reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification.

The Sulfhydryl Coupling Resin is available as a resin slurry or prealiquoted as five 2ml spin column format.

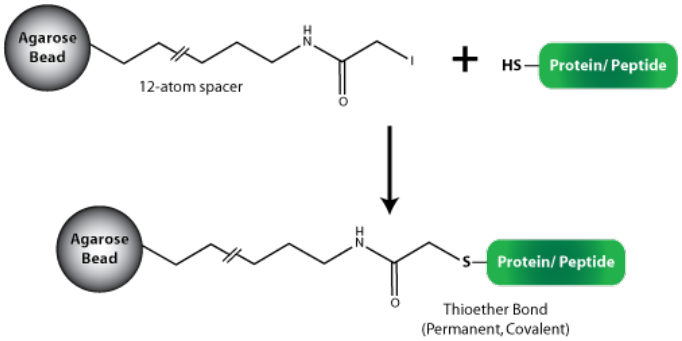


Figure 7: Sulfhydryl Coupling Resin scheme.

FEATURES

- Stable coupling of proteins and peptides, forms covalent thioether bonds
- Couples 1-2mg peptide and 2-20mg protein/ml resin

APPLICATIONS

- For the generation of affinity columns for antibody purification and other affinity chromatography

Cat. No.	Description	Size
786-794	Sulfhydryl Coupling Resin	10ml resin
786-795	Sulfhydryl Coupling Resin	50ml resin
786-796	Sulfhydryl Coupling Resin	250ml resin
786-806	Sulfhydryl Coupling Resin	5 x 2ml columns

Sulfhydryl Immobilization Kit for Proteins

For generation of protein affinity columns through free sulfhydryls

The Sulfhydryl Immobilization Kit for Proteins is a complete kit designed for the simple and efficient coupling of proteins to a solid agarose support. The Sulfhydryl Coupling Resin Columns utilizes iodoacetyl groups that specifically react with free sulfhydryls to form covalent, permanent thioether bonds. The long spacer arm reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification.

Proteins, including antibodies, must have free sulfhydryls for immobilization to the resin. A mild reducing agent, 2-Mercaptoethylamine, is supplied to reduce the hinge region disulfide bonds of antibodies, while preserving the functionally crucial disulfide bonds between the heavy and light chains.

The resulting columns can be used to study protein-protein interactions or for purification, via affinity chromatography. The columns, depending on the stability of the immobilized molecule, can be used several times without significant loss of activity.

FEATURES

- Generates 5 reusable, spin format affinity columns
- Specific conjugation through free sulfhydryls
- High Capacity: 2-40mg protein/ column
- Supplied with mild reducing agent for free sulfhydryls generation

APPLICATIONS

- Immobilize proteins to purify interacting molecules
- Immobilize antibodies in the correct orientation

Cat. No.	Description	Size
786-804	Sulfhydryl Immobilization Kit for Proteins	For 5 x 2ml columns

Sulfhydryl Immobilization Kit for Peptides

For generation of peptide affinity columns through free sulfhydryls

Sulfhydryl Immobilization Kit for Peptides is designed for the simple and efficient coupling of sulfhydryl-containing peptides to a solid agarose support. The Sulfhydryl Coupling Resin Columns utilizes iodoacetyl groups that specifically react with free sulfhydryls to form covalent, permanent thioether bonds. The long spacer arm reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification.

Peptides must have free sulfhydryls for immobilization to the resin. The supplied Protein-S-S-Reductant™ reducing agent efficiently reduces disulfide bonds and does not interfere with the iodoacetyl coupling reaction. Protein-S-S-Reductant™ offers the advantage that it does not require removal before peptide immobilization.

The resulting columns can be used for the purification of antibodies that have been raised against the specific peptide. The columns, depending on peptide stability, can be used several times.

FEATURES

- Generates 5 reusable, spin format affinity columns
- Specific conjugation through free sulfhydryls
- High Capacity: 2-4mg peptide/column

APPLICATIONS

- Immobilize peptides for antibody purification

Cat. No.	Description	Size
786-805	Sulfhydryl Immobilization Kit for Peptides	For 5 x 2ml columns

Amine Coupling Resin

The amine reactive HOOK™ Activated Agarose is 6% agarose that has been activated to generate reactive aldehyde groups. The aldehyde groups of the agarose react spontaneously with primary amines, located at the N-terminus of proteins or in lysine residues, to form intermediate Schiff Base complexes. These, in turn, are selectively reduced by reductive amination to form stable amine linkages between the agarose and the ligand.

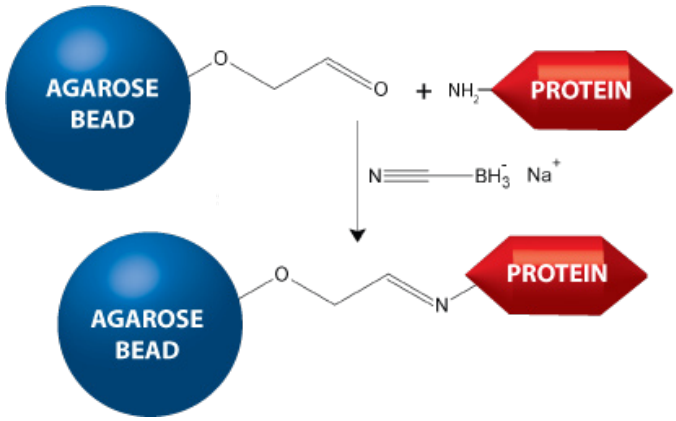


Figure 8: Scheme for the coupling of proteins to HOOK™ Activated Agarose (Amine Reactive).

The amine reactive HOOK™ Activated agarose is also supplied in a complete kit for the generation of 5 x 2ml resins. The kit is supplied with all the necessary reagents and columns.

FEATURES

- Binding capacity: 20mg protein/ml resin
- 6% cross-linked agarose

APPLICATIONS

- Coupling of proteins and peptides to agarose beads
- Suitable for antibody purification

CITED REFERENCES

Rudolph, V. et al (2008) J. Pharmacol. Exp. Ther. 327: 324

Cat. No.	Description	Size
786-066	HOOK™ Activated Agarose (Amine Reactive)	10ml resin
786-063	HOOK™ Activated Agarose (Amine Reactive) Coupling Kit	For 5 x 2ml columns

CDI Amine Reactive Resin

G-Biosciences CDI Amine Reactive Agarose consists of 6% cross-linked agarose activated with CDI (1,1'-carbonyl diimidazole) to form reactive imidazole carbamates.

The activation of the resin occurs in solvent and to maintain its activity the resin is supplied in acetone to prevent hydrolysis. Upon reaction of the resin with primary amine containing molecules, i.e. proteins, in basic (pH8.5-10) aqueous buffers the imidazole carbamates lose the imidazole group and form carbamate linkages.

CDI Amine Reactive Agarose is ideal for immobilizing peptides, small organic molecules and certain proteins and reactions can occur in organic solvent making it ideal for water-insoluble ligands.

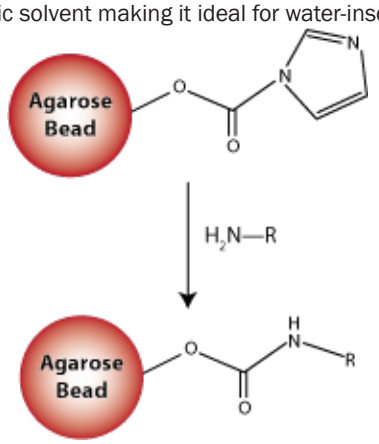


Figure 9: Scheme for the coupling of proteins to CDI Amine Reactive Agarose.

The amine reactive HOOK™ Activated agarose is also supplied in a complete kit for the generation of 5 x 2ml resins. The kit is supplied with all the necessary reagents and columns.

FEATURES

- Proven coupling chemistry
- Easy to use, no secondary coupling agents required
- Stable linkages
- Couple in inorganic buffers for insoluble molecules

APPLICATIONS

- Couple proteins and peptides
- Couple primary amine containing ligands

Cat. No.	Description	Size
786-404	CDI Amine Reactive Resin	10ml resin

Carboxyl Coupling Resin

Consists of 6% cross-linked agarose with covalent linked diaminodipropylamine (DADPA) to generate a free primary amine at the end of a long spacer arm.

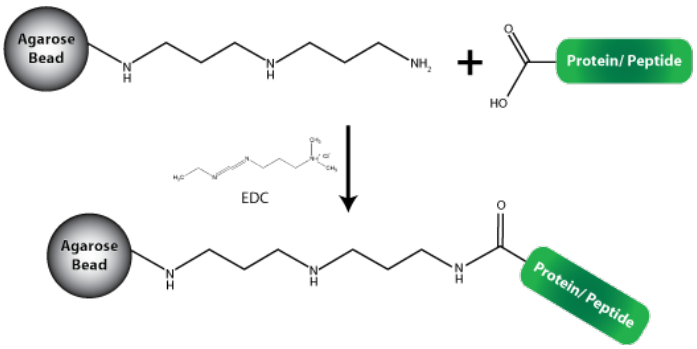


Figure 10: Carboxyl Coupling Resin scheme.

Molecules, including proteins and peptides, are covalently coupled to the free primary amines, and the stable columns are ideal for affinity purification of antibodies and other interacting partners. Molecules can be coupled to the free amine by numerous amine-reactive methods; however the use of the carbodiimide EDC allows coupling of free carboxyl groups. The resulting amide bond is highly stable and greatly reduces the chance of leaching of the affinity tag. The long spacer arm reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification.

FEATURES

- Immobilized DADPA (diaminodipropylamine)
- 6% cross-linked agarose
- Long spacer arm to limit steric hindrance
- Couple carboxyl groups

APPLICATIONS

- Couple peptides for antibody purification
- Couple peptides and proteins to purify interacting molecules

Cat. No.	Description	Size
786-797	Carboxyl Coupling Resin (Immobilized DADPA (Diaminodipropylamine))	25ml resin

SDC™ (Steroid/Drug/Compound) Immobilization

Designed for the immobilization of steroids, drugs and chemical compounds that lack primary amines, sulfhydryls, carbonyls and other common coupling groups to a solid-phase agarose support for the use in affinity purification. The kit uses Immobilized DADPA (diaminodipropylamine) resin to bind steroids, drugs and chemicals through their active hydrogens.

The coupling uses the Mannich reaction, which is described as the condensation of formaldehyde with ammonia, in the form of its salt, and another compound containing an active hydrogen. The SDC™ Immobilization kit replaces the ammonia with the primary amine on the DADPA and the active hydrogen is supplied by the steroid, drug or chemical to be coupled. Ideal for the generation of five 2ml affinity columns.

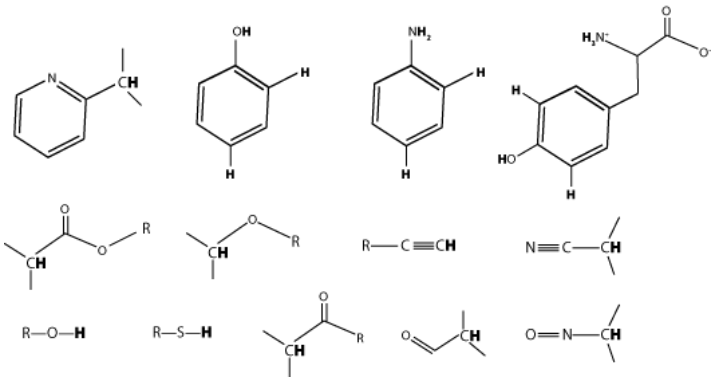


Figure 11: Active hydrogen containing compounds.

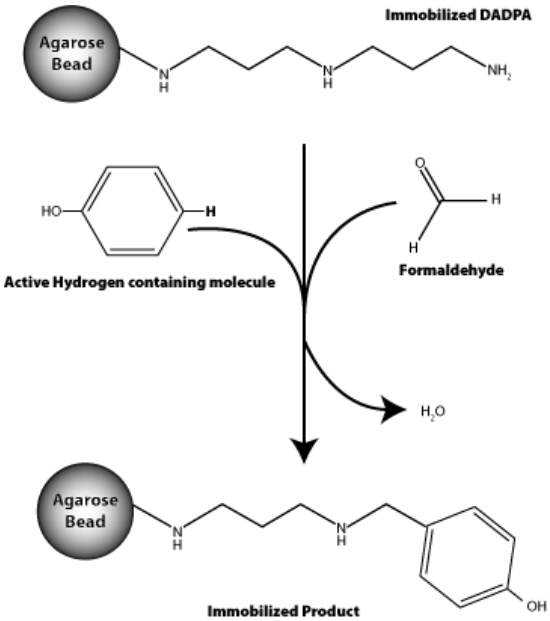


Figure 12: SDC™ (Steroid/ Drug/ Compound) Immobilization scheme.

FEATURES

- Uses Immobilized DADPA (diaminodipropylamine) resin
- Stable, covalent linkage

APPLICATIONS

- Immobilization of drugs, steroids and small metabolites through active hydrogens
- Ideal for compounds lacking primary amines, sulfhydryls, carbonyls and other common coupling groups

Cat. No.	Description	Size
786-271	SDC™ (Steroid/Drug/Compound) Immobilization	5 reactions

COATED 96-WELL PLATES

Well-Coated™ plates are available as single 96-well plates or as 12 x 8-well strips in a 96-well holder. The plates are supplied as clear, white and black plates for colorimetric, chemiluminescence and fluorescent detection systems respectively.

Well-Coated™ Protein A, Protein G & Protein A/G

Bind constant (Fc) domain of antibodies

Designed to bind the constant (Fc) region of immunoglobulins ensuring that the antigen binding domain of the antibody is orientated away from the plate, offering maximum exposure of the binding site. Protein A-G contains 4 binding sites from protein A and 2 from protein G offering maximum range of specificity and binding capacity. The immunoglobulin orientation improves the antibody capacity compared to plates that are coated directly with antibodies.

The plates are for single antibody assays and are not suitable for multiple assays (sandwich ELISAs) as the first antibody will not block all IgG binding sites and therefore false positives will occur with the second antibody. The wells are coated to a 100µl depth and are supplied pre-blocked. Clear, white and black plates are available.

See table 1 for antibody binding affinities of Protein A, Protein G and Protein A/G

FEATURES

- Protein A/G has highest specificity and capacity
- Retains antibody activity & orients antibody for maximum binding
- Reduce non-specific binding
- Binds ~4pmol rabbit IgG/well

Cat. No.	Description	Size
786-731	Well-Coated™ Protein A Coated 8-well strip plate, Clear	5 Plates
786-770	Well-Coated™ Protein A Coated 96-well plate, Black	5 Plates
786-730	Well-Coated™ Protein A Coated 96-well plate, Clear	5 Plates
786-771	Well-Coated™ Protein A Coated 96-well plate, White	5 Plates
786-733	Well-Coated™ Protein G Coated 8-well strip plate, Clear	5 Plates
786-774	Well-Coated™ Protein G Coated 96-well plate, Black	5 Plates
786-732	Well-Coated™ Protein G Coated 96-well plate, Clear	5 Plates
786-775	Well-Coated™ Protein G Coated 96-well plate, White	5 Plates
786-735	Well-Coated™ Protein A/G Coated 8-well strip plate, Clear	5 Plates
786-772	Well-Coated™ Protein A/G Coated 96-well plate, Black	5 Plates
786-734	Well-Coated™ Protein A/G Coated 96-well plate, Clear	5 Plates
786-773	Well-Coated™ Protein A/G Coated 96-well plate, White	5 Plates

Well-Coated™ Protein L

Bind kappa light chains of immunoglobulins

Designed to bind the kappa light chains of immunoglobulins without interfering with the antigen binding site. Well-Coated™ Protein L plates bind a greater range of immunoglobulin classes and subclasses compared to Protein A, G and A/G. Protein L will bind to all classes of IgG, including IgG, IgM, IgA, IgE and IgD, and binds to single chain variable fragments (scFv and Fab fragments).

The plates are for single antibody assays and are not suitable for multiple assays (sandwich ELISAs) as the first antibody will not block all IgG binding sites. The wells are coated to a 100µl depth and are supplied pre-blocked. Clear, white and black plates are offered.

FEATURES

- Retains antibody activity
- Binds to all classes of IgG, including IgG, IgM, IgA, IgE and IgD
- Reduced non-specific binding as plates are pre-blocked

TECHNICAL INFORMATION

- Only binds kappa I, III and IV in human and kappa I in mouse
- May be specific for certain kappa subgroups in other species
- Binds scFv without interfering with antigen binding
- Has weak binding affinity for rabbit immunoglobulins
- No binding affinity for bovine, goat or sheep immunoglobulins
- No binding affinity for lambda light chains

Cat. No.	Description	Size
786-737	Well-Coated™ Protein L Coated 8-well strip plate, Clear	5 Plates
786-776	Well-Coated™ Protein L Coated 96-well plate, Black	5 Plates
786-736	Well-Coated™ Protein L Coated 96-well plate, Clear	5 Plates
786-777	Well-Coated™ Protein L Coated 96-well plate, White	5 Plates

Well-Coated™ Antibody

Bind mouse or rabbit IgG antibodies

Designed to specifically bind either mouse or rabbit IgG making them suitable for binding assays using low quantities of antibodies or antibodies that denature on direct binding to polystyrene plates. Another advantage is that the specificity to IgG means purified antibodies are not essential.

Suitable for direct, indirect, competitive and sandwich assays. The wells are coated to a 100µl depth and are supplied pre-blocked. Clear, white and black plates are offered.

FEATURES

- Binds ~7pmol mouse IgG/well or ~12pmol rabbit IgG/well
- Prevents denaturation of antibodies unlike direct binding
- Species specific binding

Cat. No.	Description	Size
786-739	Well-Coated™ Antibody (goat α-mouse), 8-well strip, Clear	5 Plates
786-758	Well-Coated™ Antibody (goat α-mouse), 96-well, Black	5 Plates
786-738	Well-Coated™ Antibody (goat α-mouse), 96-well, Clear	5 Plates
786-759	Well-Coated™ Antibody (goat α-mouse), 96-well, White	5 Plates
786-741	Well-Coated™ Antibody (goat α-rabbit), 8-well strip, Clear	5 Plates
786-760	Well-Coated™ Antibody (goat α-rabbit), 96-well, Black	5 Plates
786-740	Well-Coated™ Antibody (goat α-rabbit), 96-well, Clear	5 Plates
786-761	Well-Coated™ Antibody (goat α-rabbit), 96-well, White	5 Plates

A large selection of reagents and kits for the generation of Fc, Fab and F(ab)$_2$ fragments from IgG antibodies. Utilizes optimized, immobilized papain, pepsin and ficin proteases.

Immobilized Papain

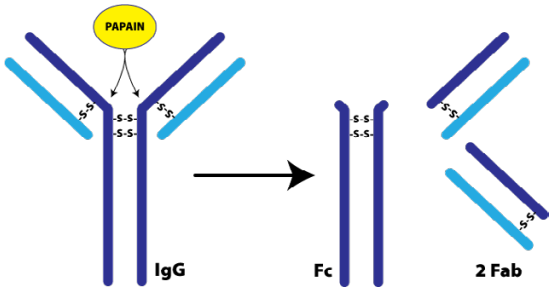


Figure 13: Digestion of Immunglobulin G with Papain.

A cysteine protease enzyme (EC 3.4.22.2) immobilized on 4% agarose, cleaves immunoglobulin G antibody molecules in the hinge region, generating three ~50kDa fragments; two Fab domains and a Fc domain. The papain-digested antibody is unable to promote agglutination, precipitation, opsonization, and lysis.

FEATURES

- Generate Fc and Fab from IgG
- Eliminates contamination with papain enzyme
- Can be used in virtually all scenarios using free papain

Cat. No.	Description	Size
786-790	Immobilized Papain	5ml Resin

Immobilized Pepsin

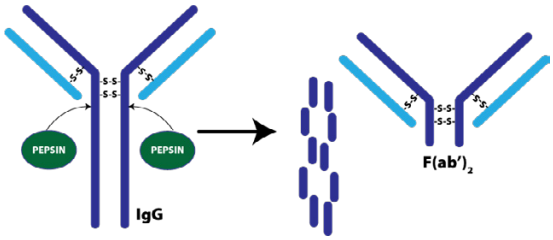


Figure 14: Digestion of Immunglobulin G with Pepsin.

A proteolytic enzyme immobilized on 4% agarose that is routinely used for the generation of F(ab')$_2$ fragments from immunoglobulin G (IgG). The pepsin has the ability to cleave the heavy chains near the hinge region. One or more of the disulfide bonds that join the heavy chains in the hinge region are preserved, so the two Fab regions of the antibody remain joined together, yielding a divalent molecule (containing two antibody binding sites), hence the designation F(ab)$_2$. The light chains remain intact and attached to the heavy chain, whereas the Fc fragment is digested into small peptides.

The Immobilized Pepsin offers the distinct advantage of eliminating enzyme contamination of the F(ab)$_2$ fragments.

FEATURES

- Generate F(ab)$_2$ fragments
- Eliminate contaminating pepsin enzyme
- Can be used in virtually all scenarios using free pepsin

Cat. No.	Description	Size
786-791	Immobilized Pepsin	5ml Resin

Immobilized Ficin

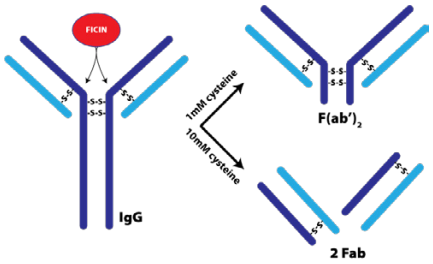


Figure 15: Digestion of Immunglobulin G with Ficin.

Ficin (or Ficain) is a cysteine protease enzyme (EC 3.4.22.3) isolated from fig latex is that has the endopeptidase activity to cleave immunoglobulin G molecules in the hinge region. Ficin is typically used to cleave mouse IgG$_1$ as this is difficult to cleave with papain and pepsin. In the presence of 1mM or 10mM cysteine, ficin generates F(ab)$_2$ and Fab fragments respectively. Immobilized Ficin is a convenient reagent for producing Fab and F(ab)$_2$ fragments as it avoids the need to remove the ficin enzyme after digestion.

FEATURES

- Generate Fab and F(ab')$_2$ fragments
- For digestion of mouse IgG1
- Eliminates contamination by Ficin

Cat. No.	Description	Size
786-793	Immobilized Ficin	5ml Resin

Fab Fragmentation

Designed for the generation and isolation of Fab fragments from IgG molecules. The kits utilize our Immobilized Papain resin. Immobilized Papain offers the advantage of generating Fab and Fc fragments without the need to remove the papain enzyme after digestion. Following papain digestion the Fab fragments are separated from undigested IgG and the Fc region with the supplied Protein A Spin Column. Protein A Resin binds the IgG and Fc molecules and the Fab are rapidly collected.

In addition, SpinOUT™ GT-600 desalting columns are supplied to ensure the initial antibody is in the optimal condition.

The Fab Fragmentation kit is optimized for mouse, rabbit and human IgG, using 0.25-4mg/ 0.5ml sample or using 25-250µg/ 125µl sample with the micro kit.

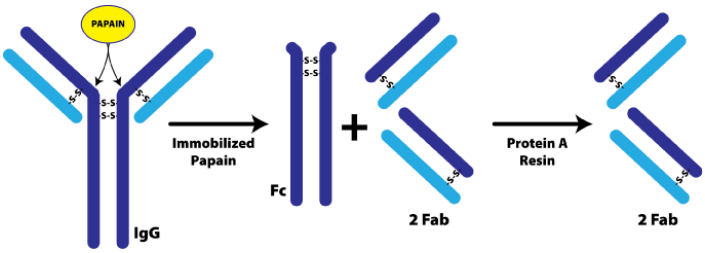


Figure 16: Fab Fragmentation scheme.

FEATURES

- Immobilized Papain ensures no enzyme contamination
- Optimized for human, mouse* and rabbit IgG
- Ready-to-use Fab fragments, with enhanced yield and purity
- Spin format for rapid purification

** For mouse IgG$_1$ fragmentation we recommend our Fab & F(ab)$_2$ Fragmentation of Mouse IgG$_1$ kits*

Cat. No.	Description	Size
786-272	Fab Preparation Kit	10 reactions
786-273	Fab Preparation Kit (Micro)	10 reactions

$F(ab)_2$ Fragmentation

The $F(ab)_2$ Fragmentation kits are designed for the generation and isolation of $F(ab)_2$ fragments from IgG molecules.

The kit utilizes our Immobilized Pepsin resin. Pepsin is a proteolytic enzyme that is routinely used for the generation of $F(ab)_2$ fragments from immunoglobulin G (IgG). The pepsin has the ability to cleave the heavy chains near the hinge region. One or more of the disulfide bonds that join the heavy chains in the hinge region are preserved, so the two Fab regions of the antibody remain joined together, yielding a divalent molecule (containing two antibody binding sites), hence the designation $F(ab)_2$. The light chains remain intact and attached to the heavy chain, whereas the Fc fragment is digested into small peptides. The Immobilized Pepsin offers the distinct advantage of eliminating enzyme contamination of the $F(ab)_2$ fragments.

Following pepsin digestion the $F(ab)_2$ fragments are separated from undigested IgG and the large Fc fragments with the supplied Protein A Spin Column. The Protein A Resin binds the IgG and Fc molecules and the $F(ab)_2$ are rapidly collected due to the spin-format design.

In addition, SpinOUT™ GT-600 desalting columns are supplied to ensure the initial antibody sample is in the optimal condition for $F(ab)_2$ Fragmentation.

The $F(ab)_2$ Fragmentation kit is optimized for mouse, rabbit and human IgG, using 0.25-4mg/ 0.5ml sample or using 25-250µg/ 125µl sample with the micro kit..

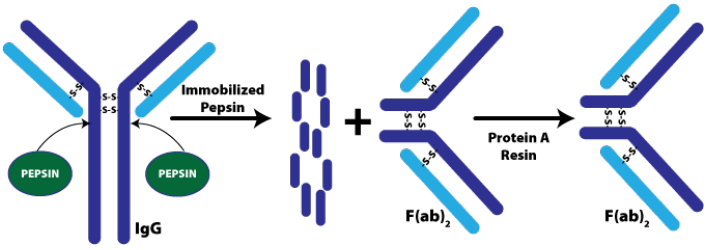


Figure 17: F(ab)2 Fragmentation scheme.

FEATURES

- Immobilized Pepsin ensures no enzyme contamination
- Optimized for human, mouse* and rabbit IgG
- Ready-to-use $F(ab)_2$ fragments, with enhanced yield and purity
- Spin format for rapid purification
- Contains all essential reagents
- Two convenient kit sizes available

** For mouse IgG_1 fragmentation we recommend our Fab & $F(ab)_2$ Fragmentation of Mouse IgG_1 kits.*

Cat. No.	Description	Size
786-274	$F(ab)_2$ Preparation Kit	10 reactions
786-275	$F(ab)_2$ Preparation Kit (Micro)	10 reactions

Fab & $F(ab)_2$ Fragmentation of Mouse IgG_1

Designed for the generation and isolation of Fab and $F(ab)_2$ fragments from mouse IgG_1 molecules.

The kit utilizes our Immobilized Ficin resin. Ficin (or Ficain) (~25,000Da) is a cysteine protease enzyme (EC 3.4.22.3) isolated from fig latex is that has the endopeptidase activity to cleave immunoglobulin G molecules in the hinge reason. Ficin has an effective range of pH4-9.5 with an optimal pH of 6.5 and cleaves bonds that involve uncharged or aromatic amino acids.

Ficin is typically used to cleave mouse IgG_1 as this is difficult to cleave with papain and pepsin. In the presence of 1-4mM or 10-20mM cysteine, ficin generates $F(ab)_2$ and Fab fragments respectively. Immobilized Ficin is a convenient reagent for producing Fab and$F(ab)_2$ fragments as it avoids the need to remove the ficin enzyme after digestion.

Following ficin digestion the fragments are separated from undigested IgG and the large Fc fragments with the supplied Protein A Spin Column. The Protein A Resin binds the IgG and Fc molecules and the Fab or $F(ab)_2$ are rapidly collected due to the spin-format design.

In addition, SpinOUT™ GT-600 desalting columns are supplied to ensure the initial antibody sample is in the optimal condition for fragmentation.

The Fab &$F(ab)_2$ Fragmentation of Mouse IgG_1 kit is optimized for mouse IgG_1, using 0.25-4mg/ 0.5ml sampleor using 25-250µg/ 125µl sample with the micro kit..

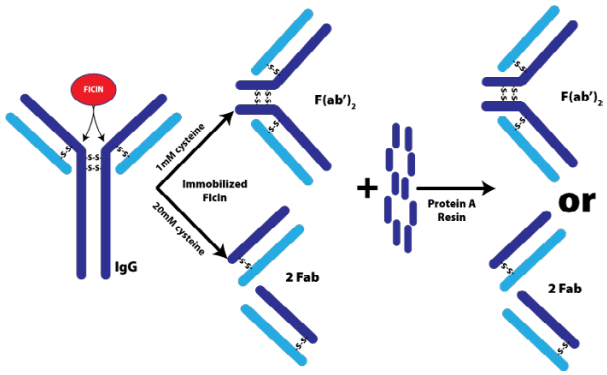


Figure 18: Fab & $F(ab)_2$ Fragmentation of Mouse IgG_1 scheme.

FEATURES

- Immobilized Ficin ensures no enzyme contamination of digestions
- Optimized for mouse IgG_1
- Results in ready-to-use Fab or F(ab)2 fragments, with enhanced yield and purity
- Spin format for rapid purification
- Contains all essential reagents
- Two convenient kit sizes available

Cat. No.	Description	Size
786-276	Mouse IgG_1 Fab & $F(ab)_2$ Preparation Kit	10 reactions
786-277	Mouse IgG_1 Fab & $F(ab)_2$ Preparation Kit (Micro)	10 reactions

FLUORESCENT DYE LABELING

The labeling of proteins with fluorescent dyes has become an important research tool in many fields. Two kits are offered for labeling virtually any protein, particularly antibodies, with either a rhodamine or fluorescein based dye.

HOOK™ Dye Labeling Kit (5/6) TAMRA-SE (Rhodamine)

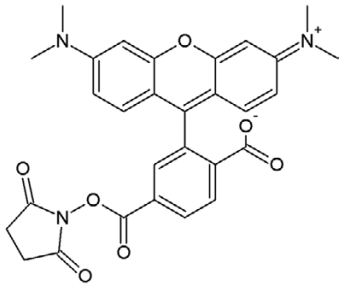


Figure 19: Structure of (5/6) TAMRA-SE.

(5/6) TAMRA-SE (5-(and-6)- Carboxytetramethylrhodamine succinimidyl ester, mixed isomers) is based on tetramethylrhodamine, one of the most common fluorophores used in the labeling of peptides, proteins, nucleic acids and nucleotides.

(5/6) TAMRA absorbs green visible light at 546nm and emits an orange-red visible light at a maximum emission of 575nm.

The NHS ester group provides the simplest and most commonly used group for labeling proteins. The succinimidyl ester group reacts with primary amines in lysine side chains and N-terminal amines forming a stable, covalent amide bond.

This kit utilizes SpinOUT™ columns for the rapid purification of dye labeled proteins.

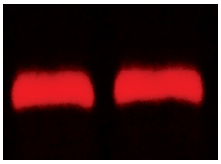


Figure 20: Visualization of TAMRA labeled BSA. 1µg (5/6) TAMRA-SE labeled BSA was resolved on a 4-20% SDS polyacrylamide gel.

FEATURES

- Dye preweighed and supplied in single use OneQuant™ vials
- Suitable for most proteins
- Utilizes SpinOUT™ desalting columns to isolate labeled protein

APPLICATIONS

- Labeling of proteins, peptides and nucleic acids with a red fluorescent dye
- Suitable for antibody labeling

CITED REFERENCES

Aktas, M. et al (2011) J. Bacteriol. 193:3473-3481

Cat. No.	Description	Size
786-142	HOOK™ (5/6) TAMRA-SE (Rhodamine) Labeling Kit	1 kit

HOOK™ Dye Labeling Kit (FITC)

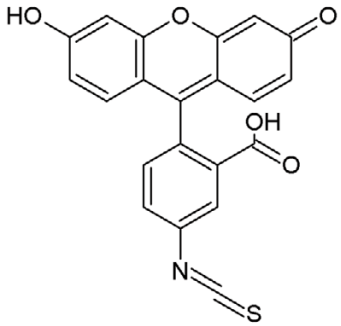


Figure 21: Structure of fluorescein isothiocyanate.

FITC (fluorescein isothiocyanate) is a commonly used fluorescent label for proteins, as it contains the groups required for conjugating to amino, sulfhydryl, imidazoyl, tyrosyl or carbonyl groups of proteins. FITC has a molecular weight of 389, and excitation and emission wavelengths of 494nm and 520nm, respectively, therefore emitting green visible light.

This kit utilizes SpinOUT™ columns for the rapid purification of dye labeled proteins.

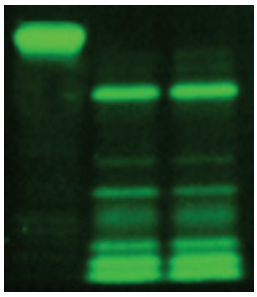


Figure 22: Visualization of FITC Labeled Casein. Lane 1: 1µg FITC labeled casein, Lane 2-3: 1µg FITC-Casein digested with 0.2µg or 0.1µg Trypsin. Samples were resolved on a 4-20% SDS polyacrylamide gel.

FEATURES

- Dye preweighed and supplied in single use OneQuant™ vials
- Suitable for most proteins
- Utilizes SpinOUT™ desalting columns to isolate labeled protein

APPLICATIONS

- Labeling of a green fluorescent dye to proteins and peptides
- Suitable for antibody labeling

Cat. No.	Description	Size
786-141	HOOK™ FITC Labeling Kit	1 kit

OneQuant™ Fluorescent Reagents

Both the fluorescent reagents (FITC and (5/6) TAMRA) are available in our *OneQuant*™ format.

The *OneQuant*™ format prevents loss of reagent due to repeated weighing. Each vial also limits exposure to light.

Cat. No.	Description	Size
786-079	OneQuant™ TAMRA	8 x 0.5mg
786-080	OneQuant™ FITC	8 x 1mg

BIOTIN LABELING

A non-radioactive labeling system

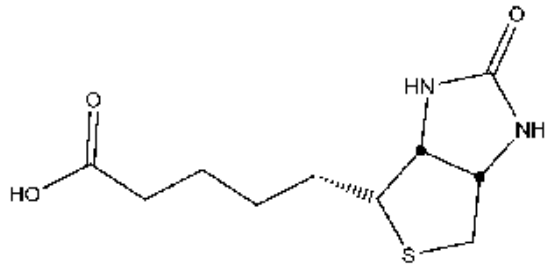


Figure 23: Structure of Biotin.

Biotin, a 244 Dalton vitamin (Vitamin H) molecule, exhibits an extraordinary binding affinity for avidin ($Ka=10^{15}M^{-1}$) and streptavidin. Biotin and avidin interaction is rapid and once the bond is established it can survive up to 3M guanidine-hydrochloride and extremes of pH. Biotin-avidin bonds can only be reversed by denaturing the avidin protein molecule with 8M guanidine-hydrochloride at pH1.5 or by autoclaving. The biotinylated molecules are efficiently probed with avidin or streptavidin conjugated to reporter molecules, such as peroxidases or phosphatases. The use of biotin for non-radioactive labeling of proteins and nucleic acids has now become an increasingly popular technique in life science research. Avidin is a glycoprotein with approximately 10% of its total mass coming from carbohydrates. Avidin has a molecular weight of 67kDa and contains four identical 128 amino acid subunits that each have a single biotin binding domain. Avidin is a basic protein with an isoelectric pH of 10-10.5 and is readily soluble in aqueous buffers containing a wide range of salt, pH, temperature and other laboratory agents. This wide range of tolerance makes avidin suitable for a wide variety of analytical applications. Streptavidin is a tetrameric protein and in many respects is similar to avidin except that it has no carbohydrate and has a slightly lower molecular weight of about 60kDa. The solubility of streptavidin (isoelectric pH5) in aqueous buffer is much lower than avidin, but the binding of streptavidin to biotin is similar to that of avidin.

Several factors must be considered when coupling a biotin reagent to a protein to ensure a successful reaction. The primary consideration is the selection of the biotinylation reagent itself. A wide range of biotin reagents are offered that have variations in their reactive groups, spacer arm lengths, solubility, membrane permeability and reversibility. All these factors must be considered and are dependent on your protein/peptide.

COUPLING FACTORS

Spacer Arms

The biotin-binding domain in avidin/ streptavidin molecules are buried 9Å below the surface and hence, the presence of bulky groups in the vicinity of the biotin-binding site may create steric hindrances and reduce the binding efficiency and the sensitivity of detection methods. Greater binding capacity can be realized by using biotin derivatives that have large spacer arms. Extended spacer arms afford the ability to overcome steric hindrances and bind deep within the binding sites of the avidin/ streptavidin molecules.

Solubility

Solubility of the HOOK™-Biotin Reagents varies greatly, with some being only soluble in organic solvents, i.e. DMSO and DMF.

Membrane Permeability

Membrane permeability has become of great interest in studies of cell surface proteins and therefore membrane trafficking and cell signaling. The HOOK™ Biotin Reagents that are not membrane permeable are excellent candidates for labeling membrane surface proteins.

Reversibility

Biotin tags are often used for protein purification, however with the biotin:avidin binding affinity being one of the strongest known it is often difficult to release the protein from the avidin. In fact, 8M guanidine at pH1.5 is often used, which has severe detrimental effects on the protein of interest. Several HOOK™ Biotin Reagents have disulfide bonds that can be reduced to release the protein of interest under mild conditions and other HOOK™ Biotin Reagents can be removed from the protein with changes in pH.

Reactive Groups

The reagents offered have numerous reactive groups that can couple to amines, sulfhydryls, carboxyls and carbohydrates. Conjugation of biotin reagents to proteins and other molecules generally does not have adverse effects on the biological properties of the target molecules, unless biotin reagents are conjugating to or modifying active residues or sites of the protein. Due to this, it is important to find an appropriate biotin reagent and optimal biotin conjugation efficiency for maintaining the functional properties of the target molecules.

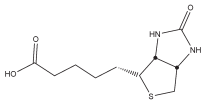
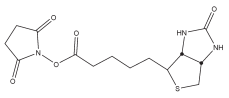
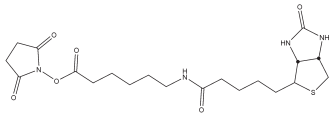
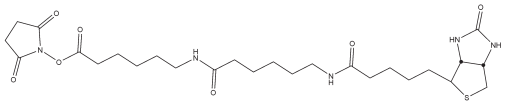
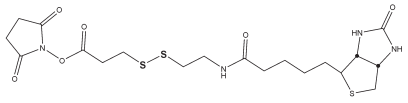
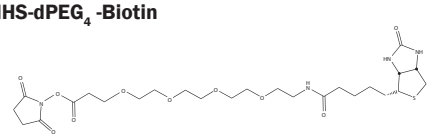
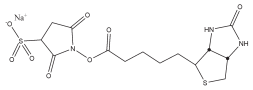
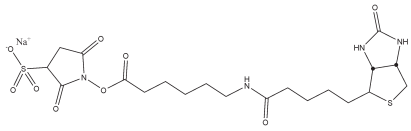
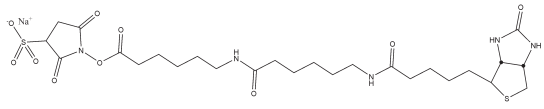
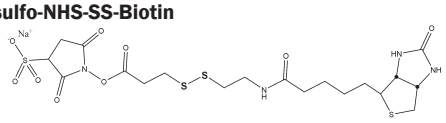
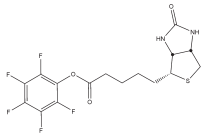
The conjugation efficiency of the reactions is dependent on the reaction groups and the buffers used for the reactions as many coupling reactions are sensitive to pH and chemical composition. The following section highlights the key features of the coupling reactions and important buffer information.

Based on the target reactive groups, biotin reagents can be divided into amine reactive, sulfhydryl reactive, carbohydrate reactive, and carboxyl reactive.

Photoreactive biotin reagents react non-specifically upon exposure to UV light and are used when no appropriate reactive target is available on the molecules.

Biotin Selection Guide

- **Reactive Group:** Determines the location of the biotin moiety
- **Membrane Permeability:** For cell surface labeling select non membrane permeable reagents
- **Cleavable:** For easy removal from immobilized avidin or streptavidin during purification
- **Reversible:** An alternative to cleavable reagents are reversible reagents
- **Steric Hinderance:** Bulky groups around the binding site may require reagents with longer spacer arms

Cat. No.	HOOK™ Biotin Reagent	Size	Molecular Weight	Spacer Arm (Å)	Reactive Group	Membrane Permeable	Water Soluble	Cleavable/ Reversible	Reaction pH
BG-00	**d-Biotin (vitamin H)** 	500mg	244.32	0					
	AMINE REACTIVE REAGENTS								
BG-01 786-083	**HOOK™-NHS-Biotin** 	50mg 8 x 2mg	341.38	13.5	NHS-ester	YES	NO	NO	7-9
BG-02	**HOOK™-NHS-LC-Biotin** 	50mg	454.54	22.4	NHS-ester	YES	NO	NO	7-9
BG-03	**HOOK™-NHS-LC-LC-Biotin** 	50mg	567.70	30.5	NHS-ester	YES	NO	NO	7-9
BG-04	**HOOK™-NHS-SS-Biotin** 	50mg	504.65	24.3	NHS-ester	YES	NO	YES	7-9
BG-05 786-700	**HOOK™ NHS-dPEG$_4$ -Biotin** 	50mg 8 x 1mg	588.67	29	NHS-ester	NO	YES	NO	7-9
BG-06 786-698	**HOOK™-sulfo-NHS-Biotin** 	50mg 8 x 1mg	443.43	13.5	Sulfo-NHS ester	NO	YES	NO	7-9
BG-07 786-084	**HOOK™-sulfo-NHS-LC-Biotin** 	50mg 8 x 1mg	556.59	22.4	Sulfo-NHS ester	NO	YES	NO	7-9
BG-08	**HOOK™-sulfo-NHS-LC-LC-Biotin** 	50mg	669.75	30.5	Sulfo-NHS ester	NO	YES	NO	7-9
BG-09 786-699	**HOOK™-sulfo-NHS-SS-Biotin** 	50mg 8 x 1mg	606.69	24.3	Sulfo-NHS ester	NO	YES	YES	7-9
BG-10	**HOOK™-PFP-Biotin** 	50mg	410.36	9.6	Pentafluorophenyl ester	YES	NO	NO	7-9

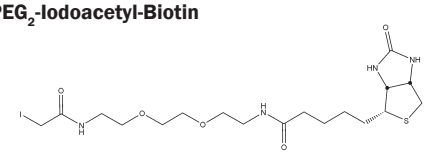
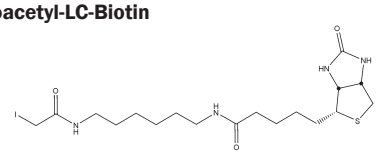
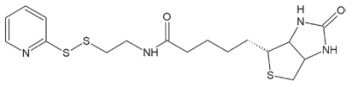
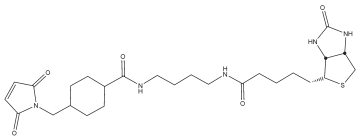
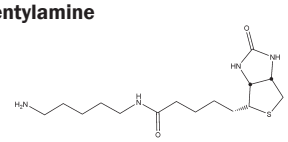
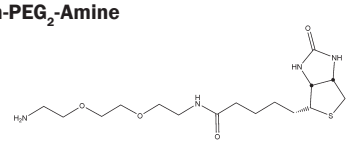
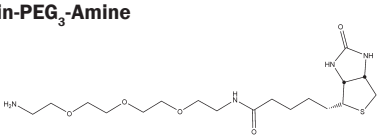
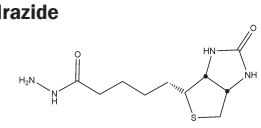
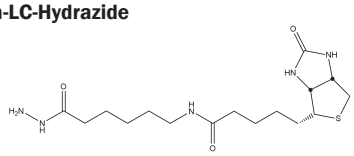
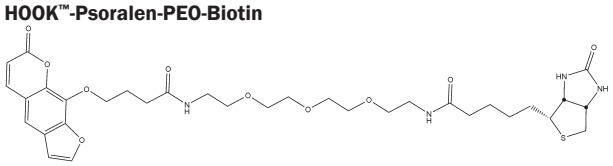
Cat. No.	HOOK™ Biotin Reagent	Size	Molecular Weight	Spacer Arm (Å)	Reactive Group	Membrane Permeable	Water Soluble	Cleavable/ Reversible	Reaction pH
SULFHYDRYL REACTIVE REAGENTS									
BG-11	**HOOK™-PEG$_2$-Iodoacetyl-Biotin** 	50mg	542.43	24.7	Iodoacetyl	NO	YES	NO	7.5-8.5
BG-12	**HOOK™-Iodoacetyl-LC-Biotin** 	50mg	510.43	27.1	Iodoacetyl	YES	NO	NO	7.5-8.5
786-085		8 x 2mg							
BG-13	**HOOK™-Biotin-PDA** 	50mg	412.60	21.1	Pyridyldithiol	YES	NO	YES	6-9
BG-14	**HOOK™-Biotin-BMCC** 	50mg	533.68	32.6	Maleimide	NO	NO	NO	6.5-7.5
CARBOXYL REACTIVE REAGENTS									
BG-15	**HOOK™-Biotin-Pentylamine** 	50mg	328.47	18.9	Amine	NO	YES	NO	4-6
BG-16	**HOOK™-Biotin-PEG$_2$-Amine** 	50mg	374.50	20.4	Amine	NO	YES	NO	4-6
BG-17	**HOOK™-Biotin-PEG$_3$-Amine** 	50mg	418.55	22.9	Amine	NO	YES	NO	4-6
CARBOHYDRATE REACTIVE REAGENTS									
BG-18	**HOOK™-Biotin-Hydrazide** 	50mg	258.34	15.7	Hydrazide	YES	NO	NO	4-6
BG-19	**HOOK™-Biotin-LC-Hydrazide** 	50mg	371.50	24.7	Hydrazide	YES	NO	NO	4-6
PHOTOREACTIVE REAGENTS									
BG-20	**HOOK™-Psoralen-PEO-Biotin** 	5mg	688.79	36.9	Psoralen	NO	YES	NO	4-6

Table 3: Biotin Selection Guide.

OneQuant™ Biotin Reagents

Several of the more commonly used HOOK™ Biotin reagents are available in our *OneQuant*™ format.

The *OneQuant*™ format prevents loss of reagent due to repeated weighing as each vial contains only 1-2mg HOOK™ Biotin Reagent.

Cat. No.	Description	Size
786-083	*OneQuant*™ HOOK™ NHS-Biotin	8 x 2mg
786-084	*OneQuant*™ HOOK™ Sulfo-NHS-LC-Biotin	8 x 1mg
786-085	*OneQuant*™ HOOK™ Iodoacetyl-LC-Biotin	8 x 2mg
786-698	*OneQuant*™ HOOK™ Sulfo-NHS-Biotin	8 x 1mg
786-699	*OneQuant*™ HOOK™ Sulfo-NHS-SS-Biotin	8 x 1mg
786-700	*OneQuant*™ HOOK™ NHS-dPEG$_4$-Biotin	8 x 1mg

HOOK™ Biotin Kits

For highly efficient labeling of proteins

HOOK™ Biotin kits come with all the necessary reagents, equipment and instructions for optimization of reaction conditions, efficient labeling, removal of unbound biotin and quantification of biotin labeling. In addition to highly efficient labeling, the HOOK™ Biotin kits offer the advantage of being supplied with SpinOUT™ desalting columns and a specific Optimizer Buffer™. These simplify the labeling process and ensure high levels of biotin labeling.

PROTEIN LABELING

Each kit is supplied with 25mg of specific HOOK™ Biotin Reagent that conjugates to proteins through amines, sulfhydryls, carboxyls or carbohydrates. The amine and sulfhydryl coupling HOOK™ Biotin Reagents couple directly to the protein through their reactive groups, however the carboxyl coupling HOOK™ Biotin Reagents require a carbodiimide crosslinker and the carbohydrate coupling HOOK™ Biotin Reagents require carbohydrate oxidation before coupling. The HOOK™ Biotin kits include EDC as the carbodiimide crosslinker in the carboxyl coupling kits and sodium meta-periodate for carbohydrate oxidation in the carbohydrate coupling kits.

In addition to the above, each HOOK™ Biotin kit contains a specific Optimizer Buffer that provides the optimal reaction conditions for each HOOK™ Biotin Reagent.

PURIFICATION

Following the labeling of the protein with the HOOK™ Biotin Reagent the unreacted biotin and other chemicals are rapidly removed from the labeled protein with the supplied SpinOUT™ columns. These columns use gel filtration to remove the by-products in <10 minutes.

BIOTIN ESTIMATION

HOOK™ BiotinQuant measures biotin using HABA [4'-hydroxyazobenzene-2-carboxylic acid] dye. HABA binds with avidin at the biotin-binding site. A characteristic color, that absorbs at 500nm, is produced (ε=35,500 $M^{-1}cm^{-1}$ expressed as per mole of HABA bound). Biotin or biotinylated agents compete with the HABA for the binding sites and the greater affinity biotin reagents displace HABA from the avidin binding sites and proportionally reduce the absorbance. The HOOK™ BiotinQuant kit is supplied with each HOOK™ Biotin Kit and is also available separately. The HABA dye is also available separately.

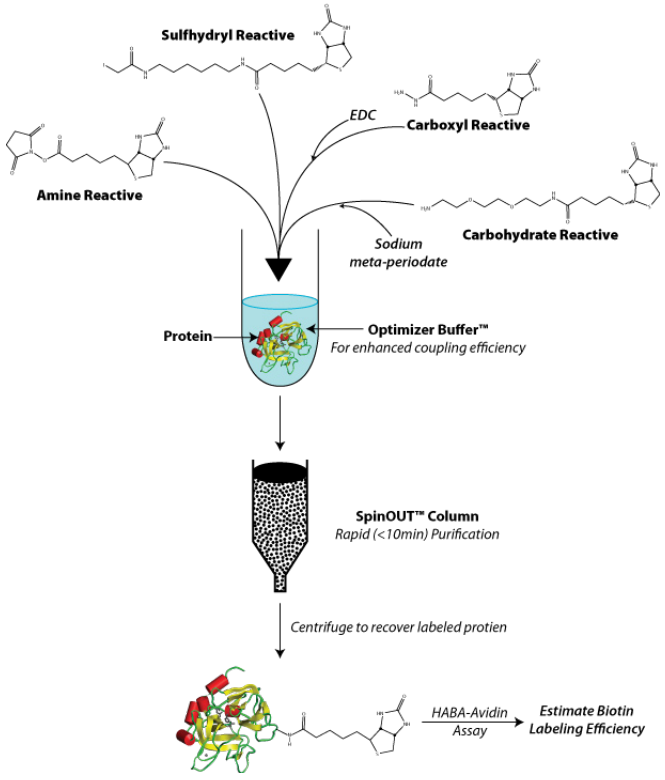


Figure 24: HOOK™ Biotin kit scheme.

FEATURES

- Optimizer Buffer™ for improved coupling efficiency
- SpinOUT™ gel filtration columns for rapid (<10 minute) purification
- Biotin assay reagents to determine level of biotin incorporation
- Labels 1-10mg protein/reaction
- Suitable for 10 coupling reactions

Cat. No.	Description	Size
BS-01	HOOK™-NHS-Biotin Kit	10 reactions
BS-02	HOOK™-NHS-LC-Biotin Kit	10 reactions
BS-03	HOOK™-NHS-LC-LC-Biotin Kit	10 reactions
BS-04	HOOK™-NHS-SS-Biotin Kit	10 reactions
BS-05	HOOK™-NHS-dPEG$_4$™-Biotin Kit	10 reactions
BS-06	HOOK™-sulfo-NHS-Biotin Kit	10 reactions
BS-07	HOOK™-sulfo-NHS-LC-Biotin Kit	10 reactions
BS-08	HOOK™-sulfo-NHS-LC-LC-Biotin Kit	10 reactions
BS-09	HOOK™-sulfo-NHS-SS-Biotin Kit	10 reactions
BS-10	HOOK™-PFP-Biotin Kit	10 reactions
BS-11	HOOK™-PEG$_2$-Iodoacetyl-Biotin Kit	10 reactions
BS-12	HOOK™-Iodoacetyl-LC-Biotin Kit	10 reactions
BS-13	HOOK™-Biotin-PDA Kit	10 reactions
BS-14	HOOK™-Biotin-BMCC Kit	10 reactions
BS-15	HOOK™-Biotin-Pentylamine Kit	10 reactions
BS-16	HOOK™-Biotin-PEG$_2$-Amine Kit	10 reactions
BS-17	HOOK™-Biotin-PEG$_3$-LC-Amine Kit	10 reactions
BS-18	HOOK™-Biotin-Hydrazide Kit	10 reactions
BS-19	HOOK™-Biotin-LC-Hydrazide Kit	10 reactions

Micro HOOK™ Biotin Kits

For highly efficient labeling of proteins

The micro HOOK™ Biotin kits are designed to label small amounts of proteins, with each kit designed for 8-10 labelings of 50-250µg protein/reaction. Each kit is supplied with all the necessary reagents for optimization of reaction conditions, efficient labeling and removal of unbound biotin. In addition to highly efficient labeling, the HOOK™ Biotin kits offer the advantage of being supplied with SpinOUT™ desalting columns and a specific Optimizer Buffer™. These simplify the labeling process and ensure high levels of biotin labeling.

PROTEIN LABELING

Each kit is supplied with 8 x 1mg single use aliquots of biotin reagent to minimize waste and degradation of the NHS ester coupling reaction group. The following HOOK™ Biotin reagents are available in the micro format:

- **HOOK™ Sulfo-NHS-Biotin**
  Amine reactive reagent, shortest spacer arm
- **HOOK™ Sulfo-NHS-LC-Biotin**
  Amine reactive reagent, longer spacer arm
- **HOOK™ Sulfo-NHS-SS-Biotin**
  Cleavable, amine reactive reagent
- **HOOK™ NHS-dPEG$_4$-Biotin**
  Amine reactive, pegylated reagent; enhances water solubility

In addition, each HOOK™ Biotin kit contains a specific Optimizer Buffer™ that provides the optimal reaction conditions.

PURIFICATION

Following the labeling of the protein with the HOOK™ Biotin Reagent the unreacted biotin and other chemicals are rapidly removed from the labeled protein with the supplied SpinOUT™ Columns. These columns use gel filtration to remove the by-products in <10 minutes.

FEATURES

- Micro kit for labeling protein primary amines
- Optimizer Buffer™ for improved coupling efficiency
- Gel filtration columns for rapid (<10 minute) purification
- Labels 50-250µg protein/reaction
- Suitable for 8-10 couplings

Cat. No.	Description	Size
786-694	HOOK™-sulfo-NHS-Biotin Kit (micro)	8-10 reactions
786-695	HOOK™-sulfo-NHS-LC-Biotin Kit (micro)	8-10 reactions
786-696	HOOK™ Sulfo-NHS-SS-Biotin Kit (micro)	8-10 reactions
786-697	HOOK™-NHS-dPEG$_4$-Biotin Kit (micro)	8-10 reactions

HOOK™ BiotinQuant

For the estimation of biotin conjugation

HOOK™ BiotinQuant measures biotin using HABA [4'-hydroxyazobenzene-2-carboxylic acid] dye. HABA binds with avidin at the biotin-binding site. A characteristic color, that absorbs at 500nm, is produced (ε=35,500 M^{-1} cm^{-1} expressed as per mole of HABA bound). Biotin or biotinylated agents compete with the HABA for the binding sites and the greater affinity biotin reagents displace HABA from the avidin binding sites and proportionally reduce the absorbance.

Cat. No.	Description	Size
BKC-01	HOOK™ BiotinQuant Kit	20 assays
BKC-03	HABA Dye	1g

HOOK™ IgG Biotinylation

Rapid antibody labeling with biotin

Designed for the efficient biotinylation of IgG molecules by first immobilizing the IgG molecules on a solid support

The HOOK™ IgG Biotinylation kits offer an advantage over standard biotinylation reactions as the immobilization of the IgG to the Nickel Chelating resin allows for the rapid removal of uncoupled biotin and therefore eliminates the need for further dialysis or desalting of the biotinylated antibody.

Two kits are available for labeling antibodies through free amines or sulfhydryls. The amine kit uses NHS-dPEG$_4$-Biotin to label free primary amines. The sulfhydryl kit uses the supplied Protein-S-S-Reductant™ to reduce the disulfide bonds of the immobilized IgG molecule. The reduced immobilized IgG molecule is then incubated with PEG$_2$-Iodoacetyl-Biotin solution to biotinylate the free sulfhydryl groups.

The advantage of a PEG (polyethylene glycol) biotinylation reagent is that the long hydrophilic spacer arm conveys its water solubility to the antibodies and have a reduced occurrence of aggregation compared to non-PEG biotinylation reactions.

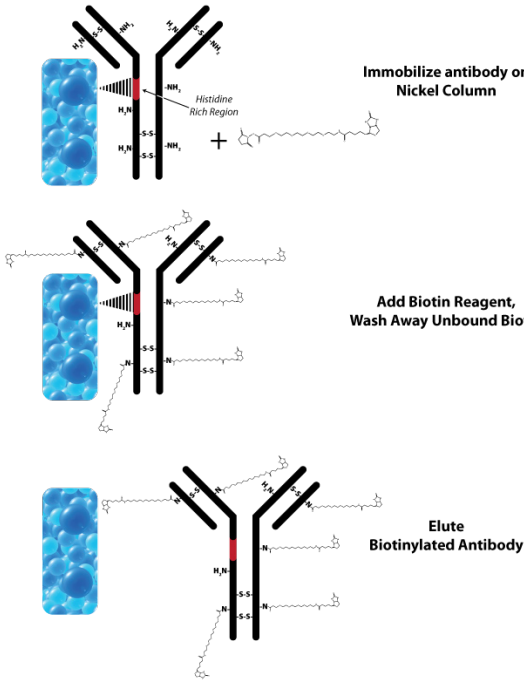


Figure 25: HOOK™ IgG Biotinylation (Amine) Scheme. The IgG antibody is first immobilized through its histidine rich domain on a nickle column. Immobilized antibody is labeled with the NHS-dPEG$_4$-Biotin reagent that reacts with primary amines. Free biotin is washed away and the biotinylated antibody is eluted with the supplied His Elution Buffer.

FEATURES

- Simpler antibody biotinylation
- Solid support technology eliminates dialysis/desalting
- Suitable for 1-10mg antibody
- PEG Biotin reagent for reduced steric hindrance and increased labeled antibody solubility

APPLICATIONS

- For the efficient and simple labeling of antibodies with biotin

Cat. No.	Description	Size
786-728	HOOK™ IgG Biotinylation (Amine)	8 reactions
786-729	HOOK™ IgG Biotinylation (Sulfhydryl)	8 reactions

BIOTIN CONJUGATION ESTIMATION

HOOK™ BiotinQuant

For the estimation of biotin conjugation

HOOK™ BiotinQuant measures biotin using HABA [4'-hydroxyazobenzene-2-carboxylic acid] dye. HABA binds with avidin at the biotin-binding site. A characteristic color, that absorbs at 500nm, is produced (ε=35,500 M^{-1} cm^{-1} expressed as per mole of HABA bound). Biotin or biotinylated agents compete with the HABA for the binding sites and the greater affinity biotin reagents displace HABA from the avidin binding sites and proportionally reduce the absorbance.

Cat. No.	Description	Size
BKC-01	HOOK™ BiotinQuant Kit	20 assays
BKC-03	HABA Dye	1g

Avidin

Affinity purified for the estimation of biotin conjugation

Avidin is a glycoprotein with approximately 10% of its total mass comes from carbohydrates. Avidin has a molecular weight of 67kDa and contains four identical 128 amino acid subunits that each have a single biotin binding domain. Avidin is a basic protein with an isoelectric pH of 10-10.5 and is readily soluble in aqueous buffers containing a wide range of salt, pH, temperature and other laboratory agents. This wide range of tolerance makes avidin suitable for a wide variety of analytical applications.

This affinity purified avidin is ideal for estimation of biotin incorporation and other applications.

Cat. No.	Description	Size
786-581	Avidin	5mg
786-582	Avidin	25mg
786-583	Avidin	100mg

HABA

A biotin estimation dye reagent.

Cat. No.	Description	Size
BKC-03	HABA	1g

BIOTIN PURIFICATION

Streptavidin Resin

High binding affinity for biotin labeled proteins & molecules

Biotin, a 244Da vitamin (Vitamin H) molecule, exhibits an extraordinary binding affinity for avidin (Ka=10^{15} M^{-1}) and streptavidin (Ka=10^{15} M^{-1}). Biotin and (strept)avidin interaction is rapid and once the bond is established it can survive up to 3M guanidine-hydrochloride and extremes of pH. Biotin-avidin bonds can only be reversed by denaturing the avidin protein molecule with 8M guanidine-hydrochloride at pH1.5 or by boiling in SDS Page Sample Loading Buffer.

Streptavidin is a tetrameric protein containing 4 biotin binding sites. Streptavidin in many respects is similar to avidin except that it has no carbohydrate and has a slightly lower molecular weight of about 60kDa. The solubility of streptavidin (isoelectric pH5) in aqueous buffer is much lower than avidin, but the binding of streptavidin to biotin is similar to that of avidin. The advantage of streptavidin is that the lack of carbohydrates significantly reduces the amount of non-specific binding.

The streptavidin used for immobilization on porous 6% crosslinked agarose is a recombinant form with a mass of 53kDa and near neutral pI. The streptavidin in covalently coupled to the agarose resulting in minimal leaching and is stable over pH2-11.

The Steptavidin Resin is designed for the single step small and large scale affinity purification of proteins and antibodies with a biotin tag. The resin can also be used for immunoprecipitations using biotin labeled antibodies. Supplied as a resin slurry or in a 1ml spin column format.

Specific Binding and Elution Buffers are also available.

The Streptavidin Resin is available as resin alone or supplied in a kit format containing:

- 5ml resin
- 100ml Streptavidin Binding/Wash Buffer (20mM $NaPO_4$, 0.15M NaCl, pH7.5)
- 100ml Streptavidin Elution Buffer (8M Guanidine.HCl pH1.5)
- 5 empty 1ml spin columns
- 5 empty <5ml gravity flow columns

The buffers are also available separately.

FEATURES

- Recombinant streptavidin covalently coupled to ~6% cross linked agarose. Minimal Leaching
- Ligand Density >1mg/ml
- Binding capacity 15-30µg biotin/ml resin

APPLICATIONS

- Immunoprecipitation with biotinylated antibodies
- Pull down assays with biotinylated proteins
- Purification of biotinylated molecules, including:
  - Proteins
  - Antibodies
  - DNA
  - Carbohydrates

Cat. No.	Description	Size
786-590	Immobilized Streptavidin Resin	2ml resin
786-390	Immobilized Streptavidin Resin	5ml Resin
786-591	Immobilized Streptavidin Resin	10ml resin
786-592	Immobilized Streptavidin Resin	5 x 1ml
786-555	Streptavidin Resin Kit	1
786-548	Streptavidin Binding Buffer	100ml
786-549	Streptavidin Elution Buffer	100ml

Avidin Resin

High binding affinity for biotin labeled proteins & molecules

Biotin, a 244Da vitamin (Vitamin H) molecule, exhibits an extraordinary binding affinity for avidin ($Ka=10^{15}\ M^{-1}$). Biotin and avidin interaction is rapid and once the bond is established it can survive up to 3M guanidine-hydrochloride and extremes of pH. Biotin-avidin bonds can only be reversed by denaturing the avidin protein molecule with 8M guanidine-hydrochloride at pH1.5 or by boiling in SDS Page Sample Loading Buffer.

Avidin is a glycoprotein with approximately 10% of its total mass coming from carbohydrates. Avidin has a molecular weight of 67kDa and contains four identical 128 amino acid subunits that each has a single biotin binding domain. Avidin is a basic protein with an isoelectric pH of 10-10.5 and is readily soluble in aqueous buffers containing a wide range of salt, pH (2-11), temperature and other laboratory agents. This wide range of tolerance makes avidin suitable for a wide variety of analytical applications. Avidin has extraordinary binding affinity for biotin ($Ka=10^{15}M^{-1}$).

The avidin in covalently coupled to the agarose resulting in minimal leaching and is stable over pH2-11.

The Avidin Resin is designed for the single step small and large scale affinity purification of proteins and antibodies with a biotin tag. The resin can also be used for immunoprecipitations using biotin labeled antibodies. Supplied as a 50% resin slurry.

Specific Binding and Elution Buffers are also available.

FEATURES

- Avidin covalently coupled to ~6% cross linked agarose. Minimal Leaching
- Binding capacity 15-20µg biotin/ml resin

APPLICATIONS

- Immunoprecipitation with biotinylated antibodies
- Pull down assays with biotinylated proteins
- Purification of biotinylated molecules, including:
  - Proteins
  - Antibodies
  - DNA
  - Carbohydrates

Cat. No.	Description	Size
786-593	Immobilized Avidin Resin	5ml Resin
786-594	Immobilized Avidin Resin	25ml Resin
786-548	Streptavidin Binding Buffer	100ml
786-549	Streptavidin Elution Buffer	100ml

Monomeric Avidin Resin

Purification & elution of biotin labeled molecules under mild elution conditions

G-Biosciences Immobilized Monomeric Avidin Resin is designed for the simple affinity chromatography purifications of proteins, antibodies and other molecules with a biotin tag. The resin consists of monomeric subunits of avidin covalently coupled to 6% cross-linked agarose, offering a stable, reusable resin for the purification of biotinylated molecules.

Monomeric avidin offers a distinct advantage over native avidin, a tetrameric molecule, and streptavidin as it has a much lower biotin binding affinity, $Kd=10^{-7}$ as opposed to $Kd=10^{-15}$ for native avidin. This lower binding affinity allows elution of molecules with mild elution buffers (2mM D-Biotin in 1X PBS), as opposed to the strong denaturing buffers (8M Guanidine•HCl, pH 1.5) used with native avidin.

The covalent attachment of monomeric avidin to the agarose ensures no detectable leaching of the avidin during biotin purification and offers a wide tolerance to chemicals. This ensures the resin can be reused at least 10 times with no loss of function.

The Immobilized Monomeric Avidin Resin is available as a 50% resin slurry or as a complete kit containing a reusable monomeric avidin column and the respective buffers for successful purification of biotinylated molecules.

FEATURES

- Monomeric avidin covalently coupled to ~6% cross linked agarose.
- Minimal Leaching
- Binding capacity »1.2mg biotinylated BSA/ml resin
- Non Denaturing: Elute biotinylated molecules with free biotin
- Reusable: Reuse the resin at least 10 times (2.5% loss of binding/ regeneration)
- Specific: Retains avidins high specificity for biotin molecules

APPLICATIONS

- Purification of biotinylated molecules, including:
  - Proteins
  - Antibodies
  - DNA
  - Carbohydrates

Cat. No.	Description	Size
786-595	Immobilized Monomeric Avidin	5ml Resin
786-596	Immobilized Monomeric Avidin	10ml Resin
786-597	Immobilized Monomeric Avidin	1 Kit

ENZYME LABELING

HOOK™ HRP PLUS Labeling

The HOOK™ HRP PLUS labeling kit is a high efficiency enzyme labeling kit for tagging proteins with horseradish peroxidase enzyme. This kit has an activated HRP that couples with high efficiency (>90%) to the numerous amine groups of proteins and is superior to the commonly used glutaraldehyde coupling chemistry.

This kit uses HOOK™ HRP PLUS, which is HRP that has been activated by the addition of reactive aldehydes. The aldehyde groups react spontaneously and at high efficiency with primary amines, located at the N-terminus of proteins or in lysine residues, to form intermediate Schiff Base complexes. These, in turn, are selectively reduced by the supplied reduction agent. Following quenching of the reaction the protein is linked to the horseradish peroxidase enzyme by stable amine linkage. The labeled protein, or antibody, can now be used for immunoblotting, ELISA and histochemical techniques.

FEATURES

- Activity is 120-200 units/mg
- Reacts with primary amines to form covalent amine bonds
- Stable for >12 months at -20°C

Cat. No.	Description	Size
786-313	HOOK™ HRP PLUS Labeling Kit	5 reactions

HOOK™ HRP Sulfo Labeling

An efficient enzyme labeling kit for tagging proteins with horseradish peroxidase (HRP) enzyme. This kit has activated HRP that couples to peptides, proteins and ligands that have free sulfhydryl groups. The maleimide activated HRP saves time as the first step of the normal two-step maleimide activation procedure is already complete, saving several hours of valuable research time.

To aid in the preparation of HRP conjugates using free sulfhydryls the kit is supplied with SATA (N-Succinimidyl S-acetylthioacetate), to add free sulfhydryls to existing amine groups, and 2-mercaptoethylamine.HCl, a mild reducing agent for conjugating HRP to immunoglobulin G (IgG) and its fragments.

FEATURES

- Reacts with free sulfhydryls to form covalent bonds
- Supplied with reagents to generate sulfhydryl groups

Cat. No.	Description	Size
786-314	HOOK™ HRP Sulfo Labeling Kit	5 reactions

HOOK™ AP Sulfo Labeling

An efficient enzyme labeling kit for tagging proteins with alkaline phosphatase enzyme. This kit has activated AP that couples to peptides, proteins and ligands that have free sulfhydryl groups. The maleimide activated AP saves time as the first step of the normal two-step maleimide activation procedure is already complete, saving several hours of valuable research time.

To aid in the preparation of AP conjugates using free sulfhydryls the kit is supplied with SATA, to add free sulfhydryls to existing amine groups, and 2-mercaptoethylamine.HCl, a mild reducing agent for conjugating AP to immunoglobulin G (IgG) and its fragments.

FEATURES

- Reacts with free sulfhydryls to form covalent bonds
- Supplied with reagents to generate sulfhydryl groups

Cat. No.	Description	Size
786-315	HOOK™ AP Sulfo Labeling Kit	5 reactions

DISPOSABLE COLUMNS

Spin Column, <0.1ml

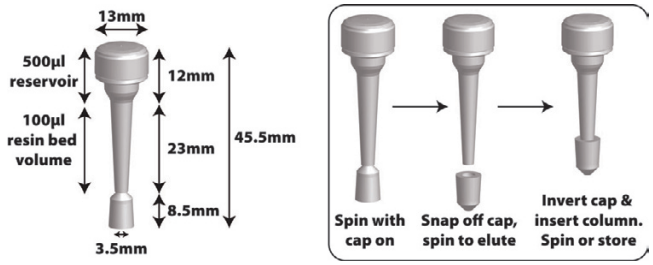


Figure 26: Spin Column, <0.1ml.

Unique design with the snap off end converting to a closure for the column for easy manipulation and use.

FEATURES

- Column volume: 600µl
- Resin volume: 5-100µl
- Filter type: Polyethylene filter, ~30µm pore size
- Fits in 1.5 and 2ml centrifuge tubes

Cat. No.	Description	Size
786-718	Spin Column, <0.1ml	25
786-719	Spin Column, <0.1ml	50

Spin Column, 1ml

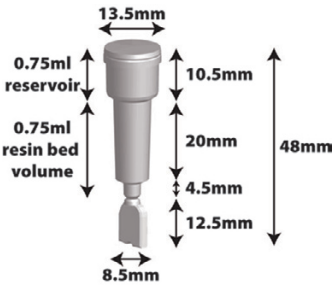


Figure 27: Spin Column, 1ml.

FEATURES

- Column volume: 1.5ml
- Resin volume: 750µl
- Filter type: Polyethylene filter, ~20µm pore size
- Cap and rubber stoppers included
- Fits in 1.5 and 2ml centrifuge tubes

Cat. No.	Description	Size
786-198	Spin Column, 1ml	10
786-720	Spin Column, 1ml	25
786-721	Spin Column, 1ml	50

Spin Column, 2ml

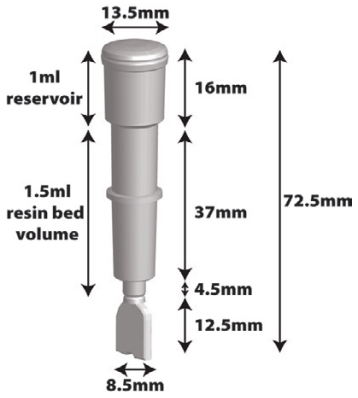


Figure 28: Spin Column, 2ml.

FEATURES

- Column volume: 2.5ml
- Resin volume: 1.5ml
- Filter type: Polyethylene filter, ~20µm pore size
- Cap and rubber stoppers included
- Fits 15ml conical centrifuge tubes

Cat. No.	Description	Size
786-722	Spin Column, 2ml	25
786-723	Spin Column, 2ml	50

Spin Column, 3ml

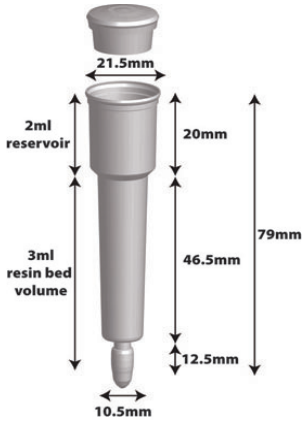


Figure 29: Spin Column, 3ml.

FEATURES

- Column volume: 5ml
- Resin volume: 3ml
- Filter type: Polyethylene filter, ~30µm pore size
- Cap and rubber stoppers included
- Fits 15ml conical centrifuge tubes

Cat. No.	Description	Size
786-724	Spin Column, 3ml	25
786-725	Spin Column, 3ml	50

Spin Column, 5ml

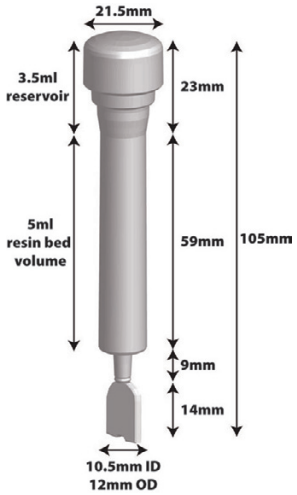


Figure 30: Spin Column, 5ml.

FEATURES

- Total volume: 8ml
- Resin volume: 5ml
- Filter type, pore size: Polyethylene filter, ~30µm pore size
- Fits 15ml conical centrifuge tubes
- Cap and rubber stoppers included

Cat. No.	Description	Size
786-726	Spin Column, 5ml	10

Spin Column, 10ml

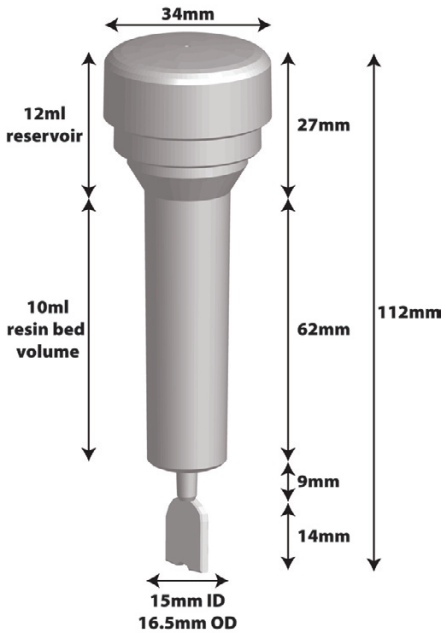


Figure 31: Spin Column, 10ml.

FEATURES

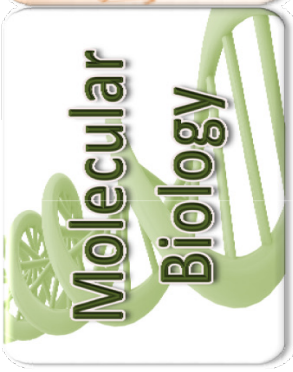
- Total volume: 22ml
- Resin volume: 10ml
- Filter type, pore size: Polyethylene filter, ~30µm pore size
- Fits 50ml conical centrifuge tubes
- Cap and rubber stoppers included

Cat. No.	Description	Size
786-727	Spin Column, 10ml	10

G-Biosciences Product Line Overview

Estimation	7 Assays	CB-X		
		Non Interfering		
		SPN		
		RED 660		
		dotMETRIC		
		BCA		
		CB		
Isolation	Extraction & Lysis	Sample Grinding		
		Lysis Buffers	Mild Denaturing	
			Strong Chaotropic	
			Specialized	
	Fractionation & Enrichment	12 Fractionation Kits		
	Sample Preparation	Dialysis (Micro)		
		Concentration		
		Contamination Removal	Desalting	
			Detergent Removal	
	Reagents	Protease Inhibitors	General Cocktails	
			Species Specific	
			Individual Inhibitors	
		Detergents		
		Chaotropes		
Detection	Electrophoresis	1D & 2D Reagents	2D Specific Kits	
			Buffers & Reagents	
		Gel Stains	Coomassie	
			Silver	
			Reversible	
	Western Blotting	1 Hour System		
		Blocking Agents	Non-Animal	
			Animal	
			Non-Protein	
		Secondary Antibodies		
		Chemiluminescence Detection		
	Mass Spectrometry	Trypsin, Mass Spec Grade		
		InGel Kits		
	Assays (ELISA)	Coated Plates		
		Blocking Agents	Non-Animal	
			Animal	
			Non-Protein	
		Secondary Antibodies		
		Detection Reagents		
Purification	Affinity Resins	6X His Tag	Nickel resin	
			Cobalt resin	
			Copper resin	
			Zinc Resin	
		GST Tag	Glutathione Resin	
		Biotin Tag	Streptavidin Resin	
		CBP Tag	Calmodulin Resin	
	Activated Resins	Sulfhydryl reactive		
		Amine reactive		
		Carboxyl reactive		
		Drug/ Steroid reactive		
	Antibody Purification	Protein A or G		
		Pearl Resin		
		Biotin		
Modification	Labeling	Fluorescent Dye		
		Enzyme (HRP/AP)		
	Crosslinkers			
	Reducing Agents			
	Alkylating Agents			
	Protein Cleavage			
	Iodination			
	Amino Acid Side Chain Modifiers			
Antibody	Production	Carrier Proteins	BSA	
			KLH	
			HyperCarrier	
	Purification	Peptide Coupling		
		Protein A or G Resin		
		Activated Resins		
		Pearl Resin		
		Thiophilic Resin		
	Fragmentation	Ficin		
		Pepsin		
		Papain		

SAM Methyltransferase	Continuous, Enzymatic Assays		
Cell Toxicity & Proliferation	Lactate Dehydrogenase (LDH)		
	SRB		
	WST-1		
Apoptosis	Caspase	Assays	
		Substrates	
		Inhibitors	
Protease	Inducers		
	Assays		
	Inhibitors		
Phosphatase			
Peroxide			
B-Galactosidase	CPRG		
	Fluorescent (MUG)		



Genomic DNA	Isolation	Tissue	
		Blood	
		Plant	
		Yeast	
		Bacteria	
		Fungi	
		Mouse Tail	
Plasmid DNA	Isolation		
	Colony Screening		
	Transformation		
Electrophoresis	Apparatus		
	Loading Dyes		
	DNA Ladders		
	Gel Extraction		
PCR	Taq		
	dNTPs		
RNA	Extraction		
	RNase Decontamination		
Yeast	Transformation		
	Plasmid Isolation		

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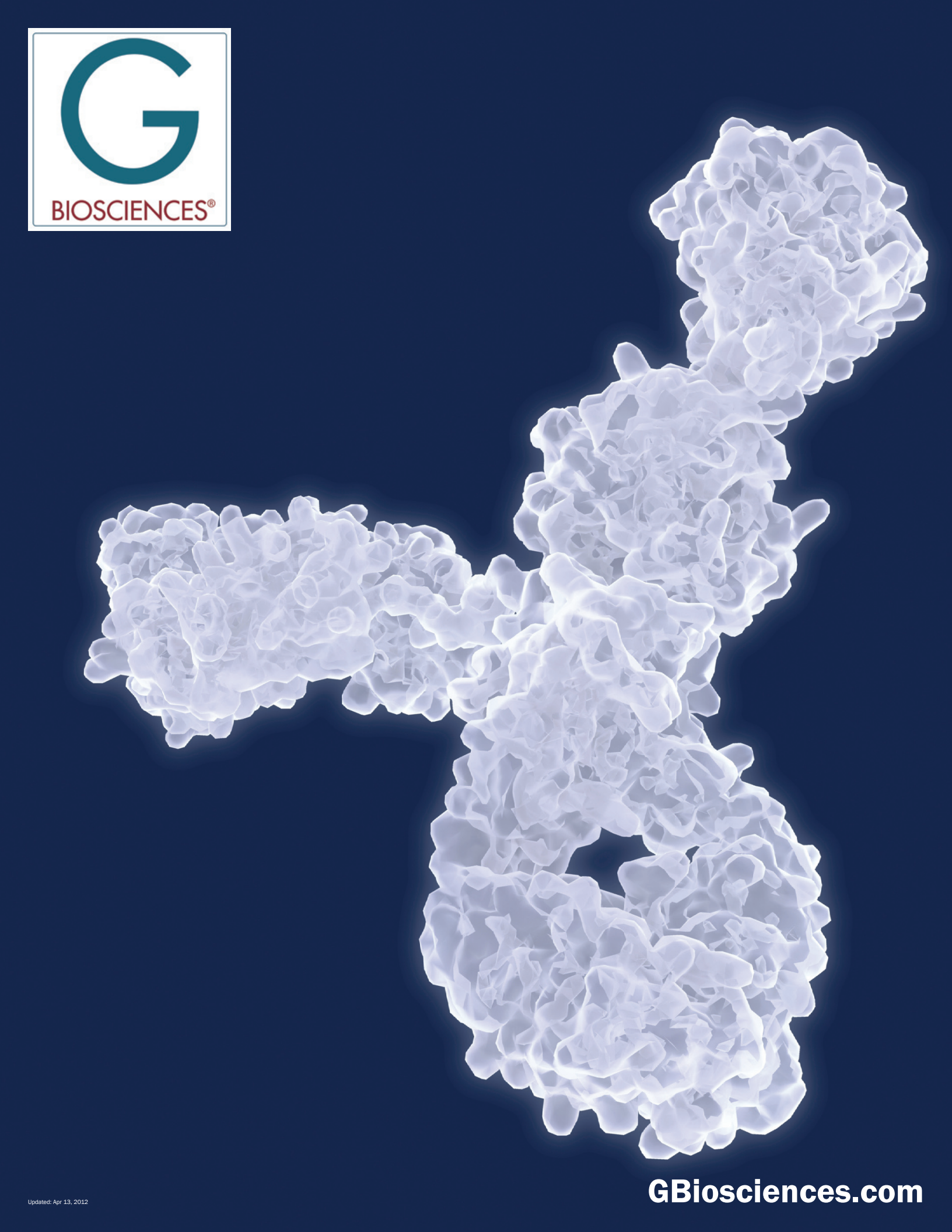
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