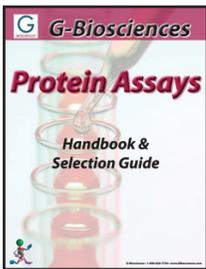




# ***G-Biosciences***

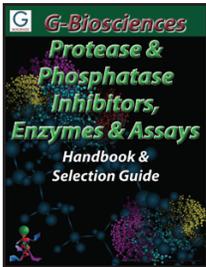
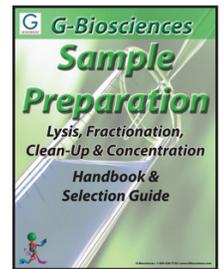
# **Protein Labeling & Conjugation**

## ***Handbook & Selection Guide***



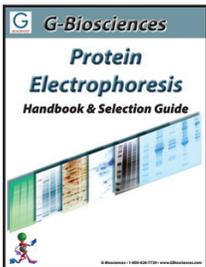
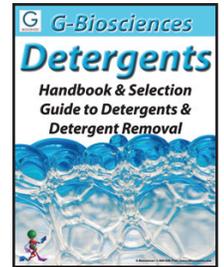
- **Protein Estimation Assays**
- **Apoptosis Assays**
- **Cytotoxicity Assays**
- **SAM Methyltransferase Assays**
- **Protease Assays**
- **Phosphatase Assays**
- **Peroxide Assay**

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



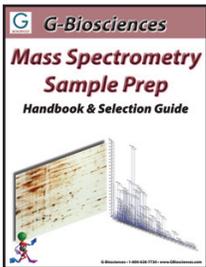
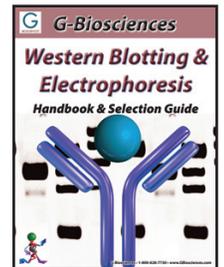
- **Protease Inhibitor Cocktails**
- **Individual Protease Inhibitors**
- **Protease Assays**
- **Proteases for Mass Spec.**
- **Sequencing Grade Proteases**

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



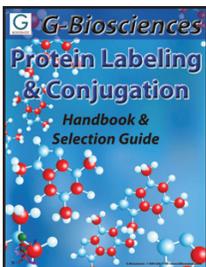
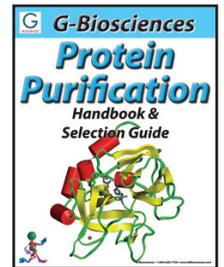
- **Gel Preparation Chemicals**
- **Protein Marker Ladders**
- **Electrophoresis Buffers**
- **Reducing & Alkylating Reagents**
- **Protein Gel Stains**

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



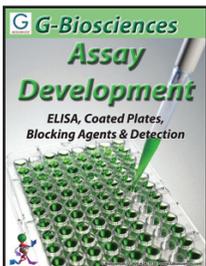
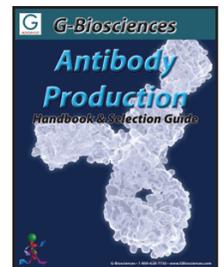
- **Protein Sample Preparation**
- **Protein Clean-Up Systems**
- **Electrophoresis Reagents**
- **Mass Spec Grade Protease**
- **InGel Digestion Kits**
- **Peptide Generation Reagents**

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



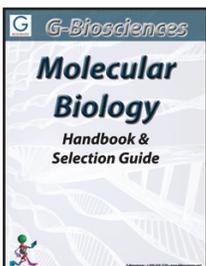
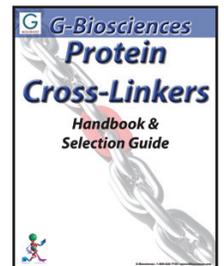
- **Biotin Labeling**
- **Cell Surface Protein Labeling**
- **Agarose Coupling Kits**
- **Fluorescent Dye Labeling Kits**
- **Enzyme Labeling Systems**

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



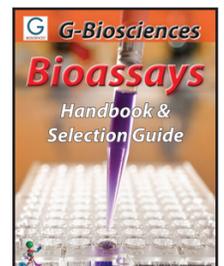
- **Coated Plates**
- **Blocking Buffers**
- **Wash Buffers**
- **Secondary Antibodies**
- **Detection Reagents**
- **Antibody Labeling Systems**

- **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**



<b>Biotin Labeling</b> .....	<b>2</b>	<b>Fluorescent Dye Labeling</b> .....	<b>15</b>
Coupling Factors .....	2	HOOK™ Dye Labeling Kit (5/6) TAMRA-SE (Rhodamine).....	15
Spacer Arms .....	2	HOOK™ Dye Labeling Kit (FITC) .....	15
Solubility .....	2	OneQuant™ Fluorescent Reagents .....	15
Membrane Permeability .....	2	<b>Labeling Accessories</b> .....	<b>16</b>
Reversibility.....	2	Coupling & Labeling Buffers.....	16
Reactive Groups .....	2	Optimizer Buffer™ .....	16
Reactive Groups .....	3	Solvents & Chemicals .....	16
Amine Reactive.....	3	DMSO & DMF.....	16
Sulfhydryl Reactive.....	3	Sodium meta-Periodate .....	16
Carboxyl Reactive .....	4	Complete Reaction System .....	16
Photoreactive .....	4	Tube-O-Reactor™ .....	16
Carbohydrate Reactive.....	4	<b>Sample Preparation &amp; Clean Up</b> .....	<b>17</b>
HOOK™ Biotin Selection Guide .....	5	Dialysis.....	17
OneQuant™ Biotin Reagents .....	7	Tube-O-DIALYZER™ .....	17
Biotin Labeling Kits .....	7	DIALYZER-Enhance™ .....	18
HOOK™ Biotin Kits .....	7	Tube-O-DIALYZER™ Accessories .....	18
Micro HOOK™ Biotin Kits .....	8	Tube-O-Array™ .....	18
HOOK™ IgG Biotinylation .....	8	Centrifuge Tube-Adapter .....	18
HOOK™ Cell Surface Protein Isolation .....	9	Tube-O-Tanks .....	18
Biotin Conjugation Estimation.....	9	Micro Dialysis Cups .....	18
HOOK™ BiotinQuant.....	9	Stirring Balls .....	18
Avidin.....	9	Floats .....	18
HABA .....	9	Contamination Removal .....	19
Biotin Purification .....	10	Spin-OUT™ .....	19
Streptavidin Resin .....	10	SpinOUT™ for PCR.....	19
Avidin Resin .....	10	Protein Concentration .....	19
Monomeric Avidin Resin .....	11	UPPA-PROTEIN-Concentrate™ .....	19
<b>Agarose Conjugation</b> .....	<b>11</b>	UPPA-I & II Pack.....	19
Sulfhydryl Reactive.....	11	OrgoSol-PROTEIN-Concentrate™ .....	20
Sulfhydryl Coupling Resin .....	11	Column-PROTEIN-Concentrate™ .....	20
Sulfhydryl Immobilization Kit for Proteins.....	12	Tube-O-CONCENTRATOR™ .....	20
Sulfhydryl Immobilization Kit for Peptides.....	12	Concentrator Solution & Powder.....	20
Amine Reactive.....	12		
Amine Coupling Resin .....	12		
CDI Amine Reactive Resin .....	13		
Carboxyl Reactive .....	13		
Carboxyl Coupling Resin .....	13		
<b>Enzyme Labeling</b> .....	<b>14</b>		
Enzyme Labeling .....	14		
HOOK™ HRP PLUS Labeling .....	14		
HOOK™ HRP Sulfo Labeling.....	14		
Active Hydrogen Reactive .....	14		
SDC™ (Steroid/Drug/Compound) Immobilization.....	14		

# Biotin Labeling

Biotin, a 244 Dalton vitamin (Vitamin H) molecule, exhibits an extraordinary binding affinity for avidin ( $K_a=10^{15}M^{-1}$ ) 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.

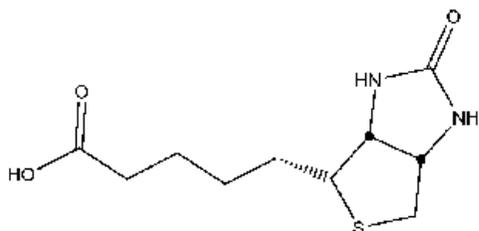


Figure 1: Structure of Biotin.

## COUPLING FACTORS

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.

## 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

This 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.

## REACTIVE GROUPS

### Amine Reactive

Amines, lysine  $\epsilon$ -amines and N-terminal  $\alpha$ -amines, are the most abundant group in protein molecules and represent the most common target for biotinylation. For example, BSA contains 59 primary amines, of which up to 35 are available on the surface of the molecules and can be reacted with amine reactive esters.

The most widely used amine reactive biotinylation reagents are the water insoluble N-hydroxysuccinimide (NHS) esters or the water soluble N-hydroxysulfosuccinimide (sulfo-NHS) esters. The addition of a charged sulfonate ( $\text{SO}_3^-$ ) on the N-hydroxysuccinimide ring of the sulfo-NHS esters results in their solubility in water ( $\sim 10\text{mM}$ ), but are not permeable to plasma membranes. The solubility and impermeability to plasma membranes makes them ideal for studying cell surface proteins as they will only react with the protein molecules on the outer surface of plasma membranes.

The reaction of the NHS and sulfo-NHS esters with amines are virtually identical leading to the formation of an amide bond and release of NHS or sulfo-NHS.

Both HOOK™-NHS-Biotin and HOOK™-sulfo-NHS-Biotin are available with various spacer arms (See Selection Guide). Also available is a cleavable form of HOOK™-sulfo-NHS-Biotin, HOOK™-sulfo-NHS-SS-Biotin, which has a disulfide bond in the spacer arm. The disulfide bond permits the cleavage of the biotin moiety from the protein, making its interaction with avidin/ streptavidin reversible. Disulfide bonds are cleaved under reducing conditions with 100mM mercaptoethanol, 30-50mM DTT, or 1% sodium borohydride.

HOOK™-PFP-Biotin is another reagent that reacts with amines and forms stable amide bonds. HOOK™-PFP-Biotin is more reactive than other NHS esters and can react with both primary and secondary amines at pH 7-9.

#### REACTION CONDITIONS

NHS esters are soluble in organic solvents and DMSO or DMF are the most commonly used, which are compatible with most proteins in a 20% solution. Sulfo-NHS ester is soluble in water, up to  $\sim 10\text{mM}$  and should only be dissolved immediately prior to use.

Reactive pH is neutral pH and above. Competing hydrolysis of the NHS esters and Sulfo-NHS esters in aqueous solution is a major concern as the rate of hydrolysis increases with increasing pH. Half-life of 2-4 hours at pH7.0 increasing to a few minutes at pH 9.0. For optimal amine coupling conditions, use Optimizer Buffer™-I.

Reaction incubation time is a few minutes to a few hours at 4-35 °C.

#### GENERAL PRECAUTIONS

Avoid buffers containing amines such as Tris or glycine.

## Sulfhydryl Reactive

Sulfhydryl reactive reagents are more specific and react only with free sulfhydryl residues (-SH or thiol groups). The side chain of the amino acid cysteine is the most common source of free sulfhydryl groups. If free sulfhydryl residues are not available, they can be generated by the reduction of disulfides (-S-S-) with reducing agents such as mercaptoethylamine, or by modifying lysine  $\epsilon$ -amines with Traut's reagent or SATA. After reduction, excess reducing agent must be removed before coupling. In addition a metal chelating agent (EDTA) (an anti-oxidant) should be used to reduce the chances of reoxidation of sulfhydryls to disulfides.

There are three different reactions employed to couple biotin reagents to sulfhydryl residues and involve either iodoacetyl, maleimide or pyridylthiol groups.

#### IDOACETYL REACTION CONDITIONS

HOOK™-PEG<sub>2</sub>-Iodoacetyl-biotin and HOOK™-Iodoacetyl-LC-biotin are both sulfhydryl reactive biotinylation reagents that react with thiol groups at pH7.5-8.5 and form stable thioether bonds. HOOK™-PEG<sub>2</sub>-Iodoacetyl-biotin is water soluble, due to its polyethylene glycol (PEG) spacer arm, while HOOK™-Iodoacetyl-LC-biotin must be dissolved in an organic solvent prior to use. Both may react with imidazoles at pH 6.9-7.0. For specific reaction with sulfhydryls, limit the reaction to pH 7.5-8.5 and the molar ratio of iodoacetyl-biotin to protein such that the concentration of biotin is only slightly higher than the sulfhydryl concentration. Iodoacetyl reaction should be performed in dark to limit the formation of free iodine, which has the potential to react with tyrosine, tryptophan, and histidine residues. For optimal iodoacetyl conjugation, we recommend Optimizer Buffer™-II.

#### MALEIMIDE REACTION CONDITIONS

HOOK™-Biotin-BMMCC is a sulfhydryl reactive reagent that contains a maleimide functional group. The maleimide group is more specific for sulfhydryl residues than iodoacetyl groups, at pH7 maleimide groups are 1000 fold more reactive toward free sulfhydryls than amines. At pH > 8.5, maleimide groups favors primary amines. Conjugation is carried out at pH 6.5-7.5 for minimizing the reaction toward primary amine. At higher pH > 8.0, hydrolysis of maleimide to maleamic acid also increases, which can compete with thiol modification. Optimizer Buffer™-III provides ideal conditions for maleimide coupling reactions.

#### PYRIDYLDITHIOL REACTION CONDITIONS

HOOK™-Biotin-PDA is a cleavable sulfhydryl reactive reagent. The reactive group is a pyridyldithiol that reacts with free sulfhydryl by disulfide exchange over a wide range of pH, forming a disulfide linkage. The optimal reaction pH is 6-9. Pyridine-2-thione is released, which absorbs light at 343nm. The coupling reaction can be monitored by measuring the absorbance of released pyridine-2-thione at 343nm. The disulfide bonds formed between HOOK™-Biotin-PDA and the protein can be cleaved with a reducing agent, generating the starting protein in its original form. This reagent is suitable for reversible applications. Optimizer Buffer™-III provides the optimized conditions.

#### GENERAL PRECAUTIONS

Remove reducing agents from the conjugation reaction. Add metal chelating agent EDTA as an anti-oxidant.

## Carboxyl Reactive

HOOK™-Biotin-Pentylamine, HOOK™-Biotin-PEG<sub>2</sub>-Amine and its long chain form, HOOK™-Biotin-PEG<sub>3</sub>-Amine, are carboxyl reactive biotinylation reagents. These agents contain terminal amines and react with carboxyl groups found at the carboxyl termini, aspartate, and glutamate side chains. The reaction is mediated by a water-soluble carbodiimide. The carbodiimide (EDC) activates the carboxyl group and reacts with the amines (-NH<sub>2</sub>) on the biotinylation agent to form an amide bond. This reaction is rapid and takes just a few minutes to complete. Under these conditions, hydrazide-derivatives of biotin reagents may also react with the carboxyls.

### REACTION CONDITIONS

The reaction is mediated by EDC, a water-soluble carbodiimide cross-linking agent. EDC activates carboxyl groups to bind with the -NH<sub>2</sub> group from the biotin derivatives. Optimizer Buffer™-IV provides the ideal buffer for EDC and other carbodiimides.

### GENERAL PRECAUTIONS

EDC may crosslink protein, decreasing EDC and/or increasing biotin reagent levels minimizing conjugation.

Avoid buffers containing amines, such as Tris or glycine, or carboxyls, such as acetate, citrate, etc. These buffers react with aldehydes, quenching the reaction.

Phosphate buffers also reduce the conjugation efficiency.

## Photoreactive

Photoreactive agents on exposure to ultraviolet light become active and bind non-specifically with neighboring molecules. Photoreactive reagents are suitable for labeling molecules that do not contain easily reactable functional groups. There are a variety of photoreactive biotinylation reagents for the labeling of proteins, peptides, nucleic acids, and other molecules. HOOK™-Psoralen-PEO-Biotin, a photoreactive reagent, reacts and labels nucleic acids and protein molecules. When reacted with nucleic acids, it cross-links with pyrimidine bases. Cross-linking does not interfere with hybridization applications.

### REACTION CONDITIONS

Photoreactive reagents contain any aryl azide group. Aryl azide groups are chemically inert until exposed to ultraviolet light. Highly reactive and short-lived aryl nitrenes are formed, which rapidly and non-specifically react with electron-rich sites by inserting into double bonds or active hydrogen bonds (insertion into C-H and N-H sites). Uncreated aryl nitrenes undergo ring expansion and become reactive toward primary amines and sulfhydryls. A wide variety of reaction buffer conditions are acceptable for photoreactive reaction, however Optimizer Buffer-I provides excellent buffer conditions.

### GENERAL PRECAUTIONS

Avoid acidic and reducing agents since they inactivate aryl azide groups.

## Carbohydrate Reactive

Some biotin reagents do not bind directly to the protein itself but conjugate to the carbohydrate residues of glycoproteins. Carbohydrate reactive biotin reagents contain hydrazides (-NH-NH<sub>2</sub>) as a reactive group. The hydrazide reactions require carbonyl groups, such as aldehydes and ketones, which are formed by oxidative treatment of the carbohydrates. Hydrazides react spontaneously with carbonyl groups, forming a stable hydrazone bond. These reagents are particularly suitable for labeling and studying glycosylated proteins, such as antibodies and receptors. HOOK™-Biotin-hydrazide and its long spacer arm equivalent, HOOK™-Biotin-LC-hydrazide, are carbohydrate reactive reagents.

### REACTION CONDITIONS

For reaction with glycoproteins, the first step is to generate carbonyl groups that react with hydrazide, under mild oxidizing conditions with sodium periodate (NaIO<sub>4</sub>). At 1mM periodate and at 0°C, sialic acid residues on the glycoproteins can be specifically oxidized converting hydroxyls to aldehydes and ketones. At higher concentrations of 6-10mM periodate, other carbohydrates in protein molecules will be oxidized. Such oxidation reactions are performed in the dark to minimize unwanted side reactions.

Aldehyde can also be generated by enzymatic reactions. For example, neuraminidase treatment will generate galactose groups from sialic acid residues on glycoproteins and galactose oxidase converts primary hydroxyl groups on galactose and N-acetylgalactosamine to their corresponding aldehydes. For coupling to carbohydrates, Optimizer Buffer™-V is recommended.

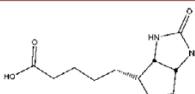
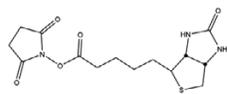
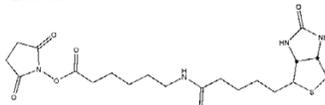
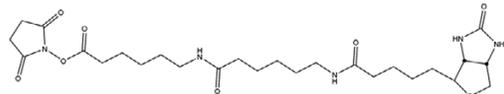
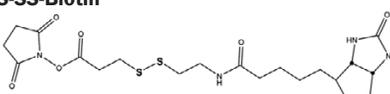
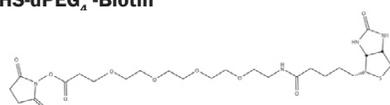
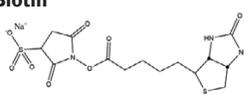
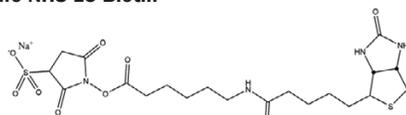
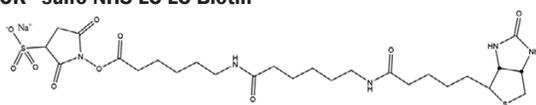
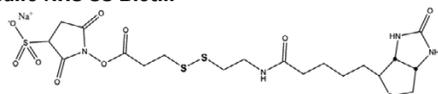
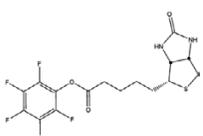
### GENERAL PRECAUTIONS

Each glycoprotein has an optimal pH for oxidation and optimal pH for the hydrazide reaction. Periodate oxidation is dependent on temperature, pH, as well as concentration. The extent of glycosylation varies for each protein; therefore, optimal condition for each protein must be determined.

Avoid buffers containing amines, such as Tris or glycine; these buffers react with aldehydes, quenching their reaction with hydrazides.

## HOOK™ 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 Hindrance:** 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	<b>d-Biotin (vitamin H)</b> 	500mg	244.32	0					
<b>AMINE REACTIVE REAGENTS</b>									
BG-01	<b>HOOK™-NHS-Biotin</b> 	50mg	341.38	13.5	NHS-ester	YES	NO	NO	7-9
786-083		8 x 2mg							
BG-02	<b>HOOK™-NHS-LC-Biotin</b> 	50mg	454.54	22.4	NHS-ester	YES	NO	NO	7-9
BG-03	<b>HOOK™-NHS-LC-LC-Biotin</b> 	50mg	567.70	30.5	NHS-ester	YES	NO	NO	7-9
BG-04	<b>HOOK™-NHS-SS-Biotin</b> 	50mg	504.65	24.3	NHS-ester	YES	NO	YES	7-9
BG-05	<b>HOOK™ NHS-dPEG<sub>n</sub> -Biotin</b> 	50mg	588.67	29	NHS-ester	NO	YES	NO	7-9
786-700		8 x 1mg							
BG-06	<b>HOOK™-sulfo-NHS-Biotin</b> 	50mg	443.43	13.5	sulfo-NHS ester	NO	YES	NO	7-9
786-698		8 x 1mg							
BG-07	<b>HOOK™-sulfo-NHS-LC-Biotin</b> 	50mg	556.59	22.4	sulfo-NHS ester	NO	YES	NO	7-9
786-084		8 x 1mg							
BG-08	<b>HOOK™-sulfo-NHS-LC-LC-Biotin</b> 	50mg	669.75	30.5	sulfo-NHS ester	NO	YES	NO	7-9
BG-09	<b>HOOK™-sulfo-NHS-SS-Biotin</b> 	50mg	606.69	24.3	sulfo-NHS ester	NO	YES	YES	7-9
786-699		8 x 1mg							
BG-10	<b>HOOK™-PPF-Biotin</b> 	50mg	410.36	9.6	Pentafluorophenyl ester	YES	NO	NO	7-9

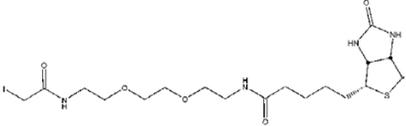
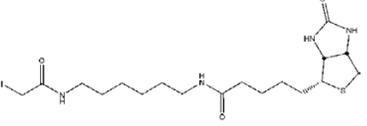
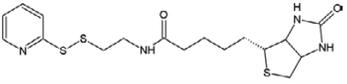
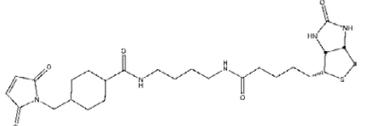
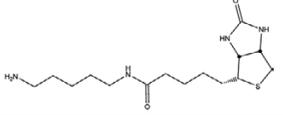
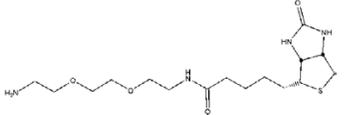
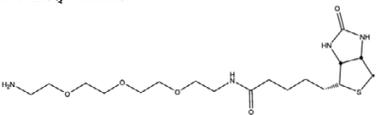
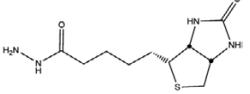
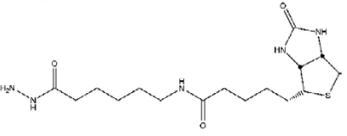
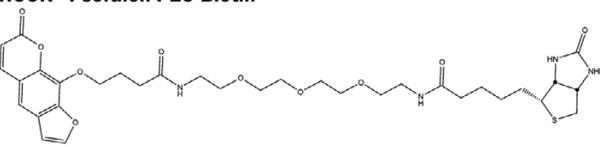
Cat. No.	HOOK™ Biotin Reagent	Size	Molecular Weight	Spacer Arm (Å)	Reactive Group	Membrane Permeable	Water Soluble	Cleavable/Reversible	Reaction pH
<b>SULFHYDRYL REACTIVE REAGENTS</b>									
BG-11	<b>HOOK™-PEG<sub>3</sub>-Iodoacetyl-Biotin</b> 	50mg	542.43	24.7	Iodoacetyl	NO	YES	NO	7.5-8.5
BG-12	<b>HOOK™-Iodoacetyl-LC-Biotin</b> 	50mg	510.43	27.1	Iodoacetyl	YES	NO	NO	7.5-8.5
786-085		8 x 2mg							
BG-13	<b>HOOK™-Biotin-PDA</b> 	50mg	412.60	21.1	Pyridyldithiol	YES	NO	YES	6-9
BG-14	<b>HOOK™-Biotin-BMMCC</b> 	50mg	533.68	32.6	Maleimide	NO	NO	NO	6.5-7.5
<b>CARBOXYL REACTIVE REAGENTS</b>									
BG-15	<b>HOOK™-Biotin-Pentylamine</b> 	50mg	328.47	18.9	Amine	NO	YES	NO	4-6
BG-16	<b>HOOK™-Biotin-PEG<sub>3</sub>-Amine</b> 	50mg	374.50	20.4	Amine	NO	YES	NO	4-6
BG-17	<b>HOOK™-Biotin-PEG<sub>6</sub>-Amine</b> 	50mg	418.55	22.9	Amine	NO	YES	NO	4-6
<b>CARBOHYDRATE REACTIVE REAGENTS</b>									
BG-18	<b>HOOK™-Biotin-Hydrazide</b> 	50mg	258.34	15.7	Hydrazide	YES	NO	NO	4-6
BG-19	<b>HOOK™-Biotin-LC-Hydrazide</b> 	50mg	371.50	24.7	Hydrazide	YES	NO	NO	4-6
<b>PHOTOREACTIVE REAGENTS</b>									
BG-20	<b>HOOK™-Psoralen-PEO-Biotin</b> 	5mg	688.79	36.9	Psoralen	NO	YES	NO	4-6

Table 1: 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 <sub>4</sub> -Biotin	8 x 1mg

## BIOTIN LABELING KITS

### 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 ( $\epsilon=35,500 \text{ M}^{-1}\text{cm}^{-1}$  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.

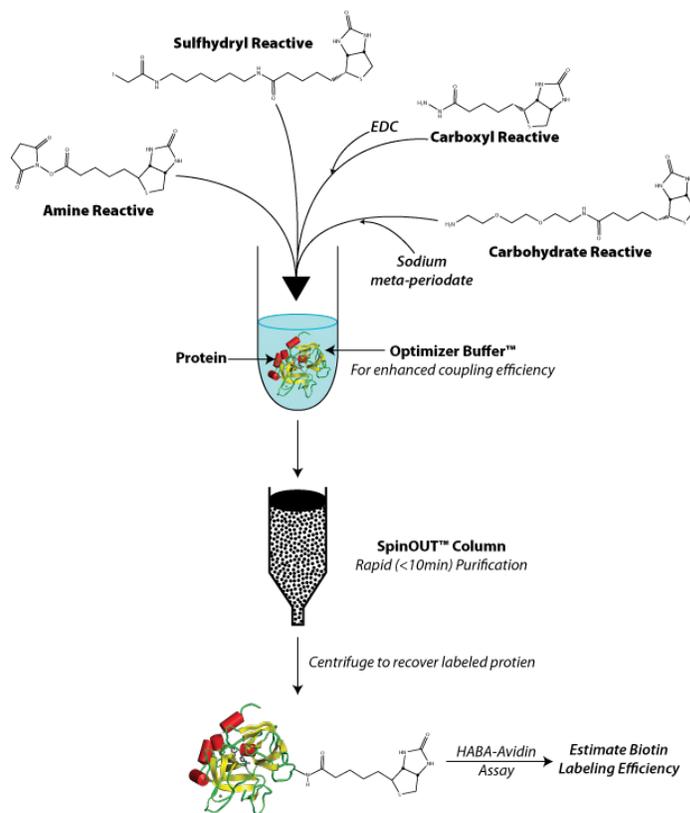


Figure 2: HOOK™ Biotin kit scheme.

#### 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 <sub>4</sub> -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 <sub>2</sub> -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-BMMCC Kit	10 reactions
BS-15	HOOK™-Biotin-Pentylamine Kit	10 reactions
BS-16	HOOK™-Biotin-PEG <sub>2</sub> -Amine Kit	10 reactions
BS-17	HOOK™-Biotin-PEG <sub>3</sub> -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<sub>4</sub>-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 <sub>4</sub> -Biotin Kit (micro)	8-10 reactions

## 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<sub>4</sub>-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<sub>2</sub>-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.

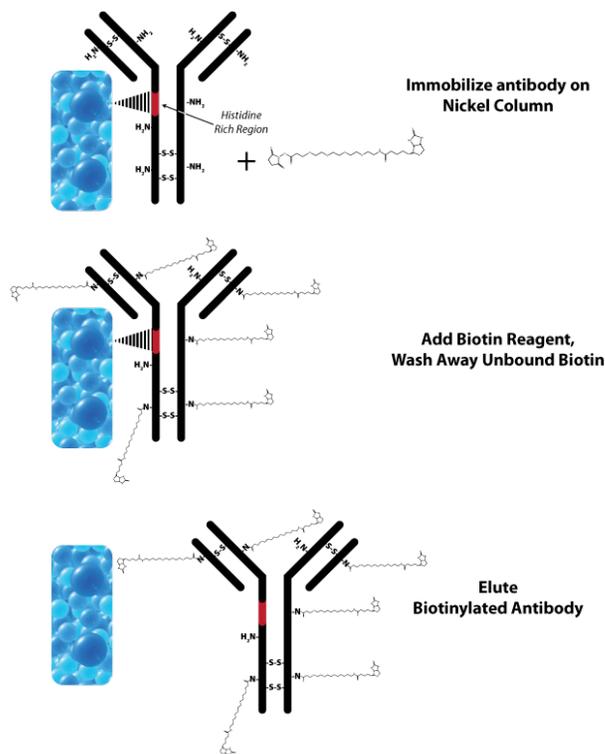


Figure 3: HOOK™ IgG Biotinylation (Amine) Scheme. The IgG antibody is first immobilized through its histidine rich domain on a nickel column. Immobilized antibody is labeled with the NHS-dPEG<sub>4</sub>-Biotin reagent that reacts with primary amines. Free biotin is washed away and the biotinylated antibody 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

## HOOK™ Cell Surface Protein Isolation

### Complete cell surface protein labeling & isolation

Uses our proven biotin labeling and purification technology in conjunction with our Mammalian Cell PE LB™ lysis buffer to conveniently label cell surface proteins and isolate them for further analysis.

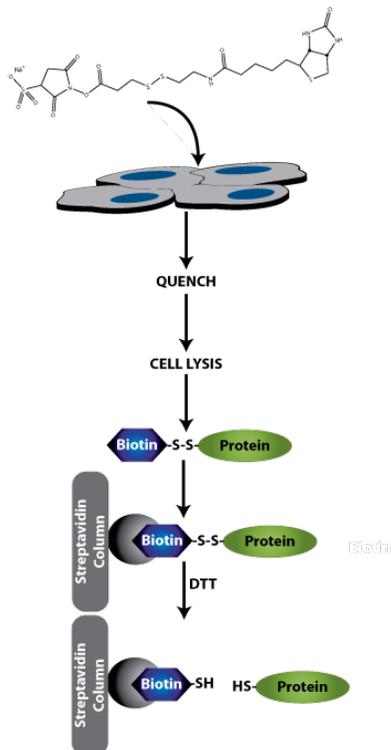


Figure 4: HOOK™ Cell Surface Protein Isolation scheme.

Mammalian cells, adherent or non-adherent, are labeled with Sulfo-NHS-SS-Biotin, an amine reactive biotinylation reagent that is soluble in water, but impermeable to plasma membranes. Sulfo-NHS-SS-Biotin has a disulfide bond in the spacer arm that permits the cleavage of the biotin moiety from the protein, making its interaction with streptavidin purification column reversible.

Cells are lysed with Mammalian Cell PE LB™ and applied to a Streptavidin agarose column. Unlabeled intracellular proteins are washed away and the biotin labeled cell surface proteins are then released by reduction of the disulfide bond with DTT.

The kit is supplied with all the necessary reagents and buffers for convenience and improved reproducibility. The kit is compatible with a wide variety of mammalian cells and can be used to compare treated and untreated cells and differences between different cell lines. This kit is supplied with sufficient reagents for five experiments, with each experiment consisting of four 90-95% confluent T-75cm<sup>2</sup> flasks.

### FEATURES

- Complete cell surface labeling & isolation kit
- Convenient; all required reagents are included
- Versatile; suitable for wide selection of mammalian cells

### APPLICATIONS

- For the isolation of cell surface proteins
- Study receptor:ligand interaction
- Study membrane trafficking

Cat. No.	Description	Size
786-316	HOOK™ Cell Surface Protein Isolation	5 Expts

## 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 ( $\epsilon=35,500 \text{ M}^{-1} \text{ cm}^{-1}$  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 ( $K_a=10^{15} M^{-1}$ ) and streptavidin ( $K_a=10^{15} M^{-1}$ ). 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 is covalently coupled to the agarose resulting in minimal leaching and is stable over pH2-11.

The Streptavidin 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 and carbohydrates

Cat. No.	Description	Size
786-584	Streptavidin, recombinant	5mg
786-585	Streptavidin, recombinant	25mg
786-586	Streptavidin, recombinant	100mg
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 ( $K_a=10^{15} M^{-1}$ ). 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 ( $K_a=10^{15} M^{-1}$ ).

The avidin is 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,  $K_d=10^{-7}$  as opposed to  $K_d=10^{-15}$  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	Kit

Activated resins have immobilized groups bound to agarose beads that can be used to generate specific affinity columns for protein, antibody and other molecule purification.

Activated resins offered include:

- Sulfhydryl Coupling Resin: Activated iodoacetyl groups for coupling free sulfhydryls
- HOOK™ Activated Agarose (Amine Reactive): Activated aldehyde groups for coupling primary amines
- Carboxyl Coupling Resin: Immobilized DADPA (Diaminodipropylamine) generates a free amine to conjugate carboxyls and other moieties with the aid of crosslinkers
- HOOK™ Activated Agarose (Carboxyl/Sulfhydryl Reactive): Aminoethyl groups with a free amine to conjugate carboxyls, sulfhydryls and other moieties with the aid of crosslinkers
- SDC™ Immobilization: Uses Immobilized DADPA (Diaminodipropylamine) for the immobilization of steroids, drugs and chemical compounds that lack primary amines, sulfhydryls, carbonyls and other common coupling groups

## SULFHYDRYL REACTIVE

### 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. 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.

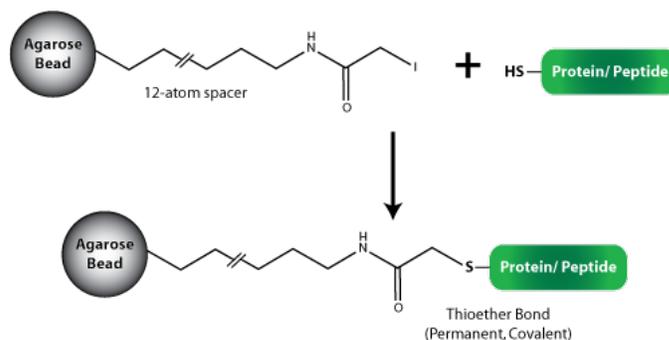


Figure 5: Sulfhydryl Coupling Resin scheme.

#### 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

# Agarose Conjugation

## 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 REACTIVE

### 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.

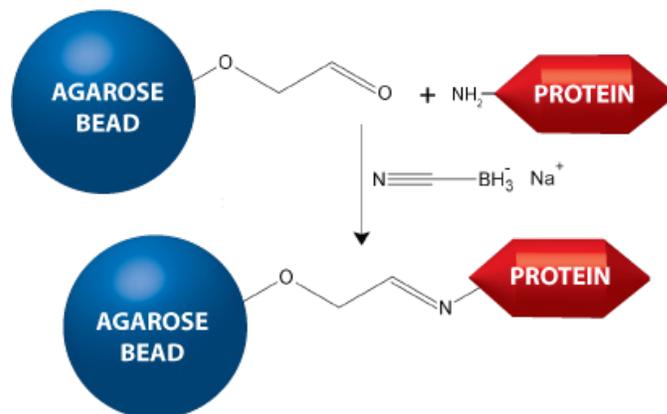


Figure 6: Scheme for the coupling of proteins to HOOK™ Activated Agarose (Amine Reactive).

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 Coupling Kit (Amine Reactive)	For 5 x 2ml columns

## CARBOXYL REACTIVE

### 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.

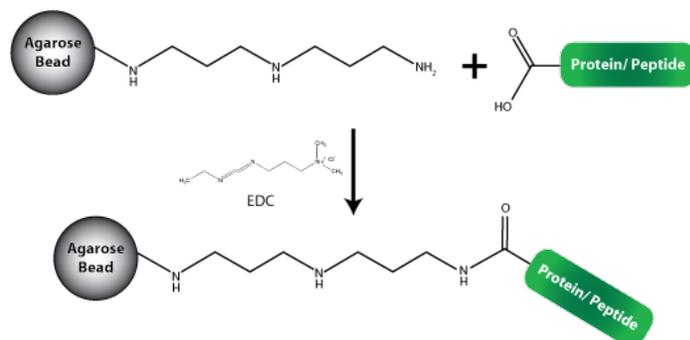


Figure 8: Carboxyl Coupling Resin scheme.

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
786-809	Carboxyl Immobilization 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.

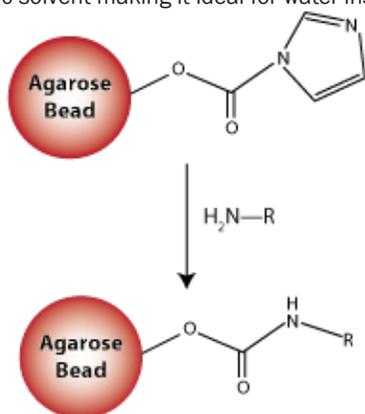


Figure 7: Scheme for the coupling of proteins to CDI Amine Reactive Agarose.

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

## ACTIVE HYDROGEN REACTIVE

### 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.

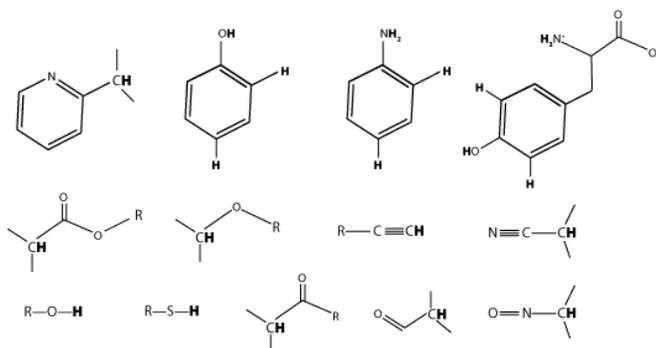


Figure 9: Active hydrogen containing compounds.

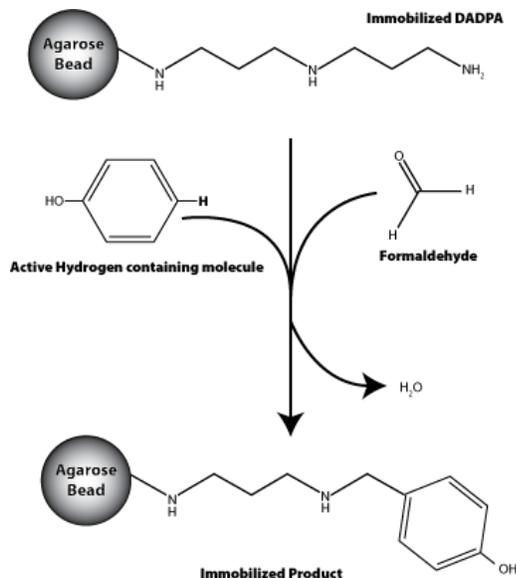


Figure 10: SDC™ (Steroid/ Drug/ Compound) Immobilization scheme.

#### 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

## 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™ Dye Labeling Kit (FITC)

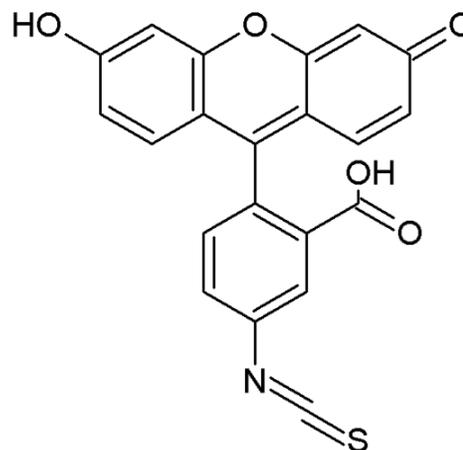


Figure 13: Structure of fluorescein isothiocyanate.

FITC (fluorescein isothiocyanate) is a commonly used fluorescent label for proteins, as it contains the groups required for conjugating to amino, sulfhydryl, imidazolyl, 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.

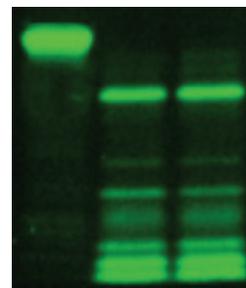


Figure 14: Visualization of FITC Labeled Casein. Lane 1: 1µg FITC labeled casein, Lane 2-3: 1µg FITC-Casein digested with 0.2µg or 0.1µg Trypsin. Samples were resolved on a 4-20% SDS polyacrylamide gel.

### 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

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)

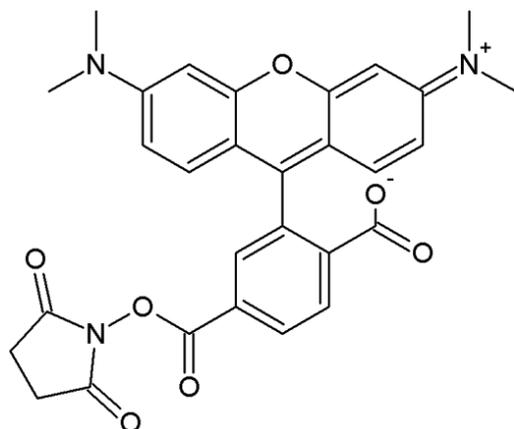


Figure 11: Structure of (5/6) TAMRA-SE.

(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.

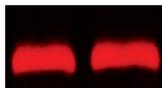


Figure 12: Visualization of TAMRA labeled BSA. 1µg (5/6) TAMRA-SE labeled BSA was resolved on a 4-20% SDS polyacrylamide gel.

### 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

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

## COUPLING & LABELING BUFFERS

### Optimizer Buffer™

#### Optimal labeling & conjugation conditions

G-Biosciences has prepared six reaction specific buffers that provide the optimal conditions for protein labeling, modification, and cross reaction. The table below highlights the reaction each buffer is specific for:

Optimizer Buffer™	Reaction Type	Reactive Group
I	Amine & Photoreactive Reactions	NHS-ester & imidoester groups
II	Sulfhydryl Reactions	Iodoacetyl groups
III	Sulfhydryl Reactions	Maleimides & pyridyl sulfides
IV	Carboxyl Reactions	Carbodiimides
V	Carbohydrate Reactions	Hydrazide groups
VI	Amine Reactions	Glyoxal groups

These buffers contain optimized concentration of buffering agents, pH, and other cofactors for specific reactions. Simply exchange the buffer of your sample with a suitable Optimizer Buffer™ and you are ready for efficient reaction. Use of SpinOUT™ or Tube-O-DIALYZER™ is recommended for buffer exchange and optimal reaction results.

Each Optimizer Buffer™ is supplied as a 5X concentrated buffer.

Cat. No.	Description	Size
BKC-04	Optimizer Buffer™-I [5X]	2 x 25ml
BKC-05	Optimizer Buffer™-II [5X]	2 x 25ml
BKC-06	Optimizer Buffer™-III [5X]	2 x 25ml
BKC-07	Optimizer Buffer™-IV [5X]	2 x 25ml
BKC-08	Optimizer Buffer™-V [5X]	2 x 25ml
BKC-09	Optimizer Buffer™-VI [5X]	2 x 25ml

## SOLVENTS & CHEMICALS

### DMSO & DMF

#### Organic solvents for HOOK™ reagents

Bottles containing anhydrous DMSO [Dimethyl sulfoxide (CH<sub>3</sub>)<sub>2</sub>SO] and DMF [N,N-Dimethylformamide (HCON(CH<sub>3</sub>)<sub>2</sub>)]. Suitable for biotinylation reaction applications.

Cat. No.	Description	Size
BKC-16	DMF, anhydrous	50ml
BKC-17	DMSO, anhydrous	50ml

### Sodium meta-Periodate

#### For the generation of active aldehydes

- A mild oxidizing agent that converts carbohydrates to activated active aldehydes
- Used in coupling to amines with cyanborohydride reduction
- Molecular weight: 213.89

Cat. No.	Description	Size
BKC-15	Sodium meta-periodate	5g

## COMPLETE REACTION SYSTEM

### Tube-O-Reactor™

#### For protein cross-linking & modification reactions

Tube-O-Reactor™ is a system that allows all the key steps of cross-linking, coupling and modification of proteins and/or nucleic acids to be performed in a single tube. This minimizes the risk of sample loss, experimental time and hands-on phases.

Most of the above reactions involve three main steps:

1. Equilibration of reaction conditions for optimized reactions
2. Subsequent reaction with target agents (i.e. cross-linkers and labels)
3. Removal of unreacted agents and by-products

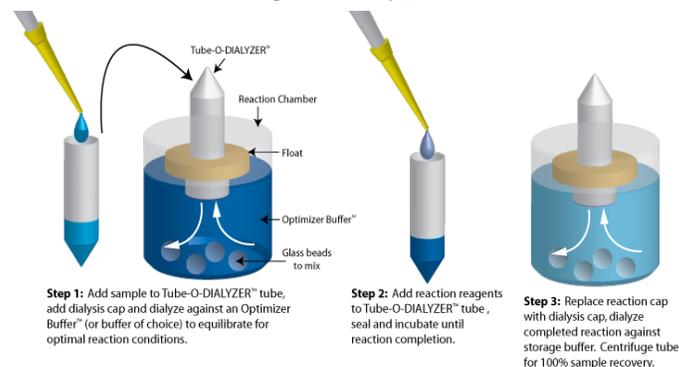


Figure 15: Tube-O-Reactor™ system.

The Tube-O-Reactor™ system is available in three MWCO sizes, 4kDa, 8kDa and 15kDa. Tube-O-Reactor™ is supplied as a Micro kit for sample sizes of 20-250µl and a Medi size for samples of 0.2-2.5ml.

Each Tube-O-Reactor™ is suitable for 5 reactions, depending on sample volumes, and is supplied with:

- 5 Medi or 5 Micro Tube-O-DIALYZER™
- 5 Floats for each size of Tube-O-DIALYZER™
- 5 Micro Dialysis Reaction Chambers
- Glass stirring balls

Cat. No.	Description	Size
786-024-4K	Tube-O-Reactor™ (Micro), 4kDa MWCO	5 units
786-024-8K	Tube-O-Reactor™ (Micro), 8kDa MWCO	5 units
786-024-15K	Tube-O-Reactor™ (Micro), 15kDa MWCO	5 units
786-027-4K	Tube-O-Reactor™ (Medi), 4kDa MWCO	5 units
786-027-8K	Tube-O-Reactor™ (Medi), 8kDa MWCO	5 units
786-027-15K	Tube-O-Reactor™ (Medi), 15kDa MWCO	5 units

Protein labeling and conjugation experiments often require the use of additional systems to remove the uncoupled labels and chemicals and other reaction by-products.

## DIALYSIS

Dialysis is a popular technique used for the exchange of buffer medium across semi-permeable membranes. Dialysis devices are available in many configurations for research applications. We offer innovative dialysis devices and accessories for processing small samples.

### Tube-O-DIALYZER™

#### Efficient dialysis with 100% sample recovery

Small sample dialysis has become a routine and popular technique in life science research. Today's major concern with dialysis devices is the loss of precious samples, due either to leaking or precipitation of samples during dialysis. A second concern is the efficiency and rate of dialysis. We manufacture a unique dialysis device that allows efficient dialysis and 100% sample recovery, even if your sample precipitates.

The unique tube format of Tube-O-DIALYZER™ allows for easy handling and manipulation. For sample recovery, just place the Tube-O-DIALYZER™ in a centrifuge and spin your sample to the bottom of the tube, ensuring 100% sample recovery, even if precipitation occurs.

The unique tube format also allows for easy sample loading, as simple as transferring your sample to a microcentrifuge tube. Tube-O-DIALYZER™ does not require the use of specialized loading devices or costly syringes and hazardous needles.

Tube-O-DIALYZER™ comes in two ideal sizes; the Micro unit allows efficient dialysis of 20-250µl samples and the Medi unit is optimized for 200µl-2.5ml samples. Both sizes are available with membranes with molecular weight cutoff (MWCO) of 1kDa, 4kDa, 8kDa, 15kDa and 50kDa. Tube-O-DIALYZER™ are available in packs of 20. Each Tube-O-DIALYZER™ is supplied with 6 floats and Tube-O-DIALYZER™ storage caps to allow storage of dialyzed samples. For added convenience, Tube-O-DIALYZER™ is also supplied as a mixed kit containing 10 Micro and 10 Medi Tube-O-DIALYZER™, along with the required floats and storage caps.

A graph representing the fast and highly efficient dialysis rate of the micro Tube-O-DIALYZER™ is shown. 100µl 5M NaCl was dialyzed against one liter deionized water. Samples were taken at specific times and the conductivity was measured. The graph demonstrates the fast efficiency of Tube-O-DIALYZER™, with 50% NaCl removed within 10 minutes.

#### APPLICATIONS

- Dialysis of small sample volumes
- Equilibrium dialysis for buffer exchange
- Concentration of samples
- Dialysis for single use applications, such as radioactive samples

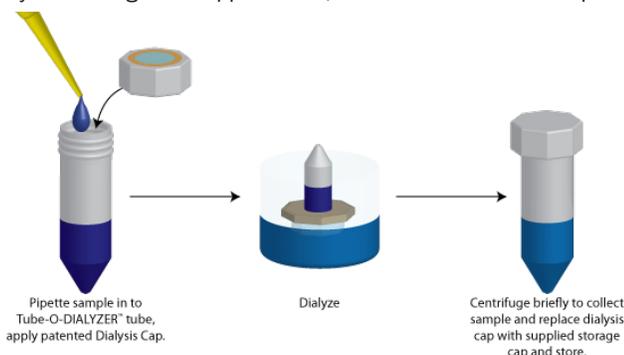


Figure 16: A summary of the Tube-O-DIALYZER™ system.

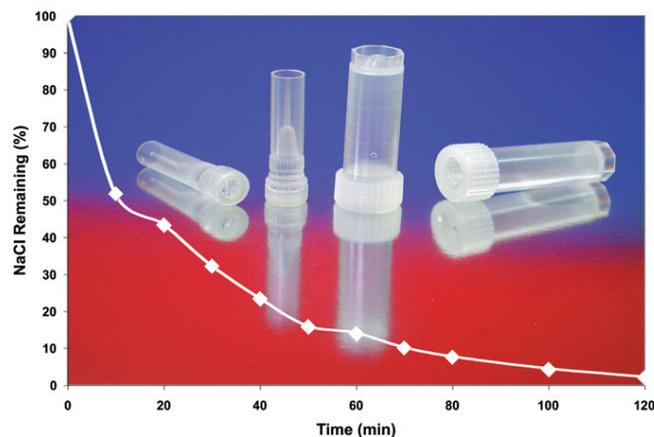


Figure 17: Tube-O-DIALYZER™ Micro (8K MWCO) Dialysis Rate. 100µl 5M sodium chloride was dialyzed against 1 liter deionized water. 50% sodium chloride is removed in the first 10 minutes.

#### CITED REFERENCES

- Rono, J. et al (2012) *Infect. Immun.* 80:1900  
 Bansal, P., et al (2009) *Biol. Reprod.* 81: 7  
 Ehmsen, K., et al (2008) *Nucleic Acids Res.* 36: 2182  
 Palmer, C., et al (2008) *PLoS ONE* 3: e2633  
 Holt, T. et al (2008) *Electrophoresis.* 26: 4486  
 Baechele, D. et al (2006) *J. Biol Chem.* 281: 5406  
 Finlay, W. et al (2005) *Clinical and Experimental Allergy.* 35: 1040  
 Ferenbach, A. et al (2005) *Nuc. Acid Res.* 33:316  
 Thomas, B. and Thekkumkara, T. (2004) *Mol. Biol of the Cell.* 15:4347  
 Roughhead, Z. et al (2003) *J. Nutr.* 133: 442  
 Tubbs, C. et al (2002) *J. Androl.* 23: 512  
 Zhang, Y. et al (2002) *Mol. Cancer Res.* 1: 122  
 Kahn, A. et al (2001) *Appl. Envir. Microbiol.* 67: 3577  
 Okamoto, H. et al (1998) *Stroke.* 29:1209

Cat. No.	Description	Size
786-610	Tube-O-DIALYZER™ Micro, 1k MWCO	20
786-610T	Tube-O-DIALYZER™ Micro, 1k MWCO	5
786-611	Tube-O-DIALYZER™ Micro, 4k MWCO	20
786-611T	Tube-O-DIALYZER™ Micro, 4k MWCO	5
786-612	Tube-O-DIALYZER™ Micro, 8k MWCO	20
786-612T	Tube-O-DIALYZER™ Micro, 8k MWCO	5
786-613	Tube-O-DIALYZER™ Micro, 15k MWCO	20
786-613T	Tube-O-DIALYZER™ Micro, 15k MWCO	5
786-614	Tube-O-DIALYZER™ Micro, 50k MWCO	20
786-614T	Tube-O-DIALYZER™ Micro, 50k MWCO	5
786-615	Tube-O-DIALYZER™ Medi, 1k MWCO	20
786-615T	Tube-O-DIALYZER™ Medi, 1k MWCO	5
786-616	Tube-O-DIALYZER™ Medi, 4k MWCO	20
786-616T	Tube-O-DIALYZER™ Medi, 4k MWCO	5
786-617	Tube-O-DIALYZER™ Medi, 8k MWCO	20
786-617T	Tube-O-DIALYZER™ Medi, 8k MWCO	5
786-618	Tube-O-DIALYZER™ Medi, 15k MWCO	20
786-618T	Tube-O-DIALYZER™ Medi, 15k MWCO	5
786-619	Tube-O-DIALYZER™ Medi, 50k MWCO	20
786-619T	Tube-O-DIALYZER™ Medi, 50k MWCO	5
786-620	Tube-O-DIALYZER™ Mixed, 1k MWCO	20
786-621	Tube-O-DIALYZER™ Mixed, 4k MWCO	20
786-622	Tube-O-DIALYZER™ Mixed, 8k MWCO	20
786-623	Tube-O-DIALYZER™ Mixed, 15k MWCO	20
786-624	Tube-O-DIALYZER™ Mixed, 50k MWCO	20

## DIALYZER-Enhance™

For the dialysis of up to 12 samples at one time

Dialysis is the process of separating molecules in solution by the difference in their rates of diffusion through a semi permeable membrane, such as dialysis tubing or Tube-O-DIALYZER™ dialysis caps. Molecules small enough to pass through the dialysis membrane move across the membrane in the direction of decreasing concentration, until an equilibrium has been reached. In order to remove the highest amount of small molecules as possible, the dialysis must be performed against large volumes of dialysis buffers and/or require frequent changes of buffer to shift the equilibrium. In fact, the approximate maximal extent a small molecule can be removed by dialysis is estimated by:  $(V_i/V_o)^{\#C}$ , where  $V_i$  is the volume inside a dialysis bag;  $V_o$  is the volume of dialysis buffer and  $\#C$  is the number of times the buffer is changed.

DIALYZER-Enhance™ is a proprietary product that when added to the dialysis buffer shifts the equilibrium resulting in the increased removal of a wide range of small molecules. The DIALYZER-Enhance™ consists of unreactive reagents that will not interfere or modify your reagents and will not cross the dialysis membrane, ensuring a pure, clean sample at the end of dialysis.

DIALYZER-Enhance™ is designed for use with our patented Tube-O-DIALYZER™ micro dialysis devices, dialysis tubing and bags for rapid and complete dialysis. 100X concentrated suspension suitable for 5 liters of dialysis buffer.

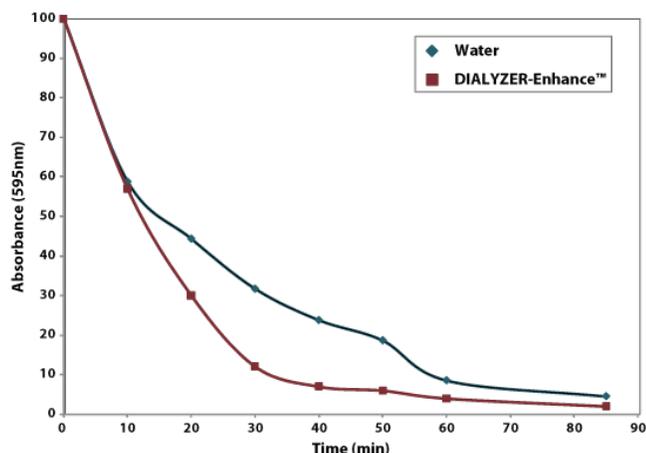


Figure 18: DIALYZER-Enhance™ reduces dialysis times. 0.5ml 5M NaCl was placed in a 8,000 MWCO Tube-O-DIALYZER™ dialyzed against 20ml water or 20ml water supplemented with DIALYZER-Enhance™.

### FEATURES

- Unreactive dialysis enhancer
- Improve dialysis rates
- Increase removal of small molecules
- 100X suspension suitable for up to 5L dialysis buffer

### APPLICATIONS

- For the enhancement of dialysis rates
- For the improved removal of small waste products
- Fully compatible with our Tube-O-DIALYZER™ range

Cat. No.	Description	Size
786-627	DIALYZER-Enhance™	50ml

## TUBE-O-DIALYZER™ ACCESSORIES

### Tube-O-Array™

For the dialysis of up to 12 samples at one time

This is a low cost system that allows for the rapid equilibration of samples in minimal buffer, requires minimal hands-on manipulation and can be used for 1-12 samples. Tube-O-Array™ consists of Tube-O-Array™ tray for supplied 12 Micro dialyzer cups. Simply add Tube-O-DIALYZER™ (supplied separately) and appropriate buffers.

#### APPLICATIONS

- Dialysis of multiple samples
- Ideal for equilibrium dialysis

### Centrifuge Tube-Adapter

For centrifugation of Medi and Micro Tube-O-DIALYZER™ in a bench top centrifuge.

### Tube-O-Tanks

Two dialysis tanks specifically designed for use with the Tube-O-DIALYZER™. Two sizes are available that are suitable for Micro and Medi size Tube-O-DIALYZER™.

### Micro Dialysis Cups

For dialysis of small sample volumes, equilibrium dialysis, dialysis of single use preparations, and other dialysis applications. The Micro Dialysis Cup has dialysis buffer capacity of 2-15 ml.

### Stirring Balls

Recommended for use with Micro Dialysis Cups for stirring dialysis buffer during dialysis. Supplied as 500 stirring balls.

### Floats

Replacement Tube-O-DIALYZER™ floats are also available separately. Floats for Tube-O-DIALYZER™ Micro and Medi sizes are available. The floats for Micro are available in two sizes: 82021-312 is designed for dialysis in Tube-O-Tanks or a beaker and 82021-336 is designed for dialysis in the Micro Dialysis Cups.

Cat. No.	Description	Size
786-145A	Tube-O-Array™	1 kit
786-145	Tube-O-DIALYZER™ Centrifuge Tube Adapter	2
786-145D	Tube-O-Tanks (Small)	1
786-145E	Tube-O-Tanks (Large)	1
786-145C	Micro Dialysis Cups	12
786-145B	Stirring Balls	500
786-141F	Tube-O-DIALYZER™ Floats (Micro)	6
786-149	Tube-O-DIALYZER™ Floats (Micro for Dialysis Cups)	12
786-142F	Tube-O-DIALYZER™ Floats (Medi)	6

## CONTAMINATION REMOVAL

### Spin-OUT™

#### For desalting and buffer exchange

The SpinOUT™ GT-600 and GT-1200 columns are versatile, spin-format columns for the desalting and buffer exchange of protein and nucleic acid solutions ranging from 5µl through to 4ml sample volumes. The SpinOUT™ columns are available in two MWCO sizes. Simply apply the sample and then centrifuge to recover protein/nucleic acids with the column retaining >95% of the salts and small molecules (<1,000Da).

Spin-OUT™ GT-600 is for the purification of proteins >6kDa and nucleic acids larger than 10bp.

Spin-OUT™ GT-1200 is for the purification of proteins >30kDa and removal of molecules >1,500Da.

#### FEATURES

- 5 sizes available for sample volumes of 5µl to 4ml
- Spin format for rapid purification

#### CITED REFERENCES

Taggert, C. et al (2005) *J. Exp. Med.* 202: 1659  
 Tripodi, K et al (2005) *Plant Physiol.* 139: 969

Cat. No.	Description	Size	Resin Bed Volume	Sample Load Volume
786-703	SpinOUT™ GT-600	25 columns	0.1mL	0.005 - 0.02mL
786-170	SpinOUT™ GT-600	10 columns	1mL	0.05 - 0.1mL
786-171	SpinOUT™ GT-600	10 columns	3mL	0.1 - 0.5mL
786-704	SpinOUT™ GT-600	5 columns	5mL	0.5 - 2mL
786-705	SpinOUT™ GT-600	5 columns	10mL	0.5 - 4mL
786-706	SpinOUT™ GT-1200	25 columns	0.1mL	0.005 - 0.02mL
786-172	SpinOUT™ GT-1200	10 columns	1mL	0.05 - 0.1mL
786-173	SpinOUT™ GT-1200	10 columns	3mL	0.1 - 0.5mL
786-707	SpinOUT™ GT-1200	5 columns	5mL	0.5 - 2mL
786-708	SpinOUT™ GT-1200	5 columns	10mL	0.5 - 4mL

### SpinOUT™ for PCR

SpinOUT™ PCR is for the cleaning of PCR products. PCR-20 removes contaminating products from PCR products, including <20bp primers, dNTPs and salts. PCR-32 removes PCR products from <32bp primers, dNTPs and salts. For more information see the DNA Clean Up & Concentration section.

Cat. No.	Description	Size
786-174	SpinOUT™ PCR-20	10 columns
786-175	SpinOUT™ PCR-32	10 columns

## PROTEIN CONCENTRATION

### UPPA-PROTEIN-Concentrate™

#### Rapid precipitation & concentration

UPPA PROTEIN-Concentrate™ uses a proprietary reagent, UPPA™ (Universal Protein Precipitation Agent), to quantitatively concentrate dilute protein samples as low as 1ng/ml. Concentration is not affected by the presence of common laboratory agents, including detergents and chaotropes. After precipitation, the sample is washed to remove salts and other interfering agents; complete recovery of sample is produced. Protein samples have conductivity of ~50µS and ~100% recovery.

UPPA PROTEIN-Concentrate™ kit is available as a Micro kit for concentrating up to 10ml of dilute protein solutions; and a Medi Kit for concentrating up to 30ml of dilute protein solutions, either as a single or multiple procedures.

#### FEATURES

- Concentrate as dilute as 1ng/ml
- Removes non-protein agents
- Low conductivity, ~ 50µS
- 100% sample recovery

#### APPLICATIONS

- For concentrating proteins for running gels, raising antibodies, protein purification, protein assays, and other applications
- This kit contains an acidic component and may not be suitable for those proteins which may lose some of their biological activities when precipitated

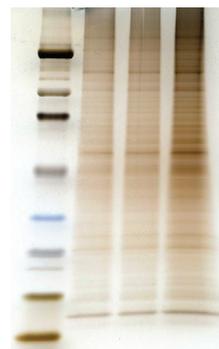


Figure 19: Concentration of dilute mouse liver lysate. Lane 1: Protein Marker; Lane 2: 20µl dilute protein (0.05µg/µl). Lane 3: 20µl original sample treated with UPPA-PROTEIN-Concentrate™ and resuspended in 20µl. Lane 4: 40µl original sample treated with UPPA-PROTEIN-Concentrate™ and resuspended in 20µl. Comparing lanes 2 and 3 shows that there is 100% protein recovery and lane 4 shows the actual concentration of a sample.

#### CITED REFERENCES

Singleton, D. et al (2004) *Microbiol.* 150: 285  
 Morisawa, G. et al (2000) *Plant Cell.* 12: 1903

Cat. No.	Description	Size
786-120	UPPA-PROTEIN-Concentrate™ (Micro)	For 10ml sample
786-121	UPPA-PROTEIN-Concentrate™ (Medi)	For 30ml sample

### UPPA-I & II Pack

UPPA™ (Universal Protein Precipitation Agent) is offered separately for the concentration of dilute (>1ng/ml) protein solutions. Concentration of proteins with UPPA™ is unaffected by chaotropes, detergents or common laboratory reagents.

Cat. No.	Description	Size
786-122	UPPA™-I & II Pack	For >80ml

For further details, visit [GBiosciences.com](http://GBiosciences.com)

# Sample Preparation & Clean Up

## OrgoSol-PROTEIN-Concentrate™

### Preserve biological activity during concentration

The OrgoSol-PROTEIN-Concentrate™ kit precipitates protein with a proprietary solvent buffer, OrgoSol™. The OrgoSol™ buffer has been specifically developed for efficient precipitation of protein solutions with minimal disruption to the protein structure and therefore maintains the biological activity of most proteins.

The kit has been extensively tested for the concentration of a wide selection of enzymatic proteins without the loss of their biological activity and for ~100% protein recovery. The kit is designed to precipitate up to 5ml protein solution.

The method involves mixing a protein solution with the OrgoSol™ Buffer followed by incubation, which results in quantitative precipitation of the protein. The precipitated protein is suspended in a smaller volume of an appropriate buffer and the concentrated protein solution is ready for use.

### FEATURES

- Precipitates enzyme proteins without loss of activity
- Uses a proprietary organic solvent buffer
- Recovery ~100%

### CITED REFERENCES

Olbro, M. et al (2002) PNAS. 99: 6737

Cat. No.	Description	Size
786-125	OrgoSol-PROTEIN-Concentrate™	For 5ml protein

## Column-PROTEIN-Concentrate™

### For larger volumes of dilute protein solutions

The Column-PROTEIN-Concentrate™ kit has been specifically developed for concentration of those proteins that cannot be concentrated by precipitation. The kit is based on a proprietary Protein Binding Resin that binds and immobilizes any protein in a low salt buffer between pH 2-12. The binding capacity is ~0.5mg protein/ml Protein Binding Resin.

The immobilized protein is spin-eluted in a small volume of specifically formulated elution buffer, giving several fold effective concentration. The method is gentle and protects protein from denaturation during the concentration process.

Suitable for concentration of a total of 4mg protein in either single or multiple procedures. request further information for concentration of >5mg protein.

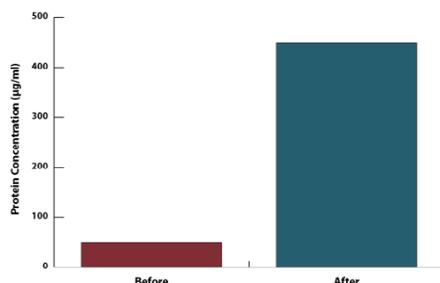


Figure 20: Column concentration of dilute proteins. 1ml dilute protein (50µg/ml) was concentrated with Column-PROTEIN-Concentrate™ to a final volume of 110µl with a concentration of 450µg/ml.

### FEATURES

- Spin column format for concentration of proteins
- Reusable protein binding resin (capacity ~0.5mg protein/ml)
- Maintains and protects biological activity of proteins
- Recovery ~ 100%

Cat. No.	Description	Size
786-126	Column-PROTEIN-Concentrate™	For 4mg protein

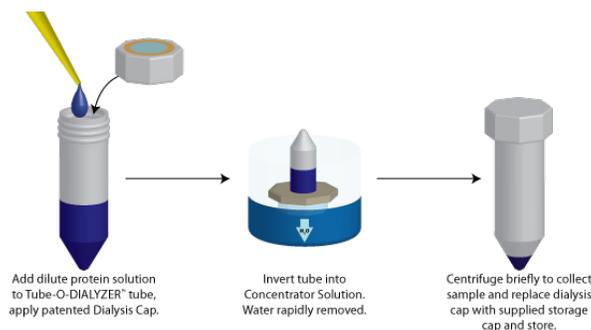
## Tube-O-CONCENTRATOR™

### Rapid concentration of proteins >1kDa without protein precipitation

Tube-O-CONCENTRATOR™ is a versatile concentration device that utilizes a novel, water absorbing, liquid polymer and our patented Tube-O-DIALYZER™ for the rapid concentration of dilute protein solutions with zero protein loss. The unique tube design of Tube-O-DIALYZER™ ensures that 100% sample is recovered; simply place the entire device in a bench top centrifuge and spin for a few seconds.

The Tube-O-CONCENTRATOR™ solution is a liquid polymer that rapidly absorbs water through the dialysis membrane in the Tube-O-DIALYZER™ cap, which retains all molecules with a molecular weight >1kDa.

Tube-O-CONCENTRATOR™ is available in two sizes for concentrating sample volumes of up to 250µl (Micro) or 2.5ml (Medi).



### FEATURES

- Rapid concentration of >1kDa proteins without protein precipitation
- Combination of a unique tube format dialysis device and a water absorbing liquid polymer
- Suitable for up to 250µl or 2.5ml protein solutions
- Zero protein loss, even if protein precipitation occurs

Cat. No.	Description	Size
786-625	Tube-O-CONCENTRATOR™ for 20-250µl	5 concentrations
786-626	Tube-O-CONCENTRATOR™ for 0.2-2.5ml	5 concentrations

## Concentrator Solution & Powder

### Concentrate proteins by dialysis

Concentrator Solution is a novel liquid polymer for the rapid concentration of dilute protein solutions with zero loss, using dialysis. Simply transfer your dilute protein solution to a dialysis bag or dialysis device, such as our patented Tube-O-DIALYZER™ and dialyze against the concentrator solution. The water will be rapidly removed through the dialysis membrane, which also retains your protein of interest and prevents the high molecular weight liquid polymer entering your solution. Once the desired volume of your solution is achieved, quickly rinse the excess concentrator solution from the dialysis bag/membrane and recover your sample.

Concentrator Powder is a high molecular weight polymer which will not migrate across the dialysis membrane. Simply transfer your dilute protein solution to a dialysis bag or dialysis device, such as our patented Tube-O-DIALYZER™ and then cover the membrane with Concentrator Powder. Concentrator Powder rapidly absorbs water from the sample and reduces the sample volume.

Cat. No.	Description	Size
786-143	Concentrator Solution	125ml
786-144	Concentrator Powder	150g

# G-Biosciences Product Line Overview

## Protein Research

- Estimation
- Isolation
- Detection
- Purification
- Modification
- Antibody

## BioAssays

- SAM Methyltransferase
- Cell Toxicity & Proliferation
- Apoptosis
- Protease
  - Phosphatase
  - Peroxide
- B-Galactosidase

## Molecular Biology

- Genomic DNA
- Plasmid DNA
- Electrophoresis
- PCR
- RNA
- Yeast

- 7 Assays
- Extraction & Lysis
  - Fractionation & Enrichment
- Sample Preparation
- Reagents
- Electrophoresis
- Western Blotting
- Mass Spectrometry
- Assays (ELISA)
- Affinity Resins
- Activated Resins
- Antibody Purification
- Labeling
  - Crosslinkers
  - Reducing Agents
  - Alkylating Agents
  - Protein Cleavage
  - Iodination
  - Amino Acid Side Chain Modifiers
- Production
- Purification
- Fragmentation

- Continuous, Enzymatic Assays
- Lactate Dehydrogenase (LDH)
  - SRB
  - WST-1
- Caspase
  - Inducers
  - Assays
  - Inhibitors

- CPRG
- Fluorescent (MUG)

- Isolation
  - Colony Screening
  - Transformation
  - Apparatus
  - Loading Dyes
  - DNA Ladders
  - Gel Extraction
  - Taq
  - dNTPs
  - Extraction
  - RNase Decontamination
  - Transformation
  - Plasmid Isolation

- CB-X
- Non Interfering
- SPN
- RED 660
- dotMETRIC
- BCA
- CB
- Sample Grinding
- Lysis Buffers
- 12 Fractionation Kits
  - Dialysis (Micro)
  - Concentration
- Contamination Removal
- Protease Inhibitors
  - Detergents
  - Chaotropes
- 1D & 2D Reagents
- Gel Stains
  - 1 Hour System
- Blocking Agents
  - Secondary Antibodies
  - Chemiluminescence Detection
  - Trypsin, Mass Spec Grade
  - InGel Kits
  - Coated Plates
- Blocking Agents
  - Secondary Antibodies
  - Detection Reagents
- 6X His Tag
  - GST Tag
  - Biotin Tag
  - CBP Tag
  - Sulphydryl reactive
  - Amine reactive
  - Carboxyl reactive
  - Drug/ Steroid reactive
  - Protein A or G
  - Pearl Resin
  - Biotin
  - Fluorescent Dye
  - Enzyme (HRP/AP)

- Mild Denaturing
- Strong Chaotropic
- Specialized

- Desalting
- Detergent Removal
- General Cocktails
- Species Specific
- Individual Inhibitors

- 2D Specific Kits
- Buffers & Reagents
- Coomassie
- Silver
- Reversible

- Non-Animal
- Animal
- Non-Protein

- Non-Animal
- Animal
- Non-Protein

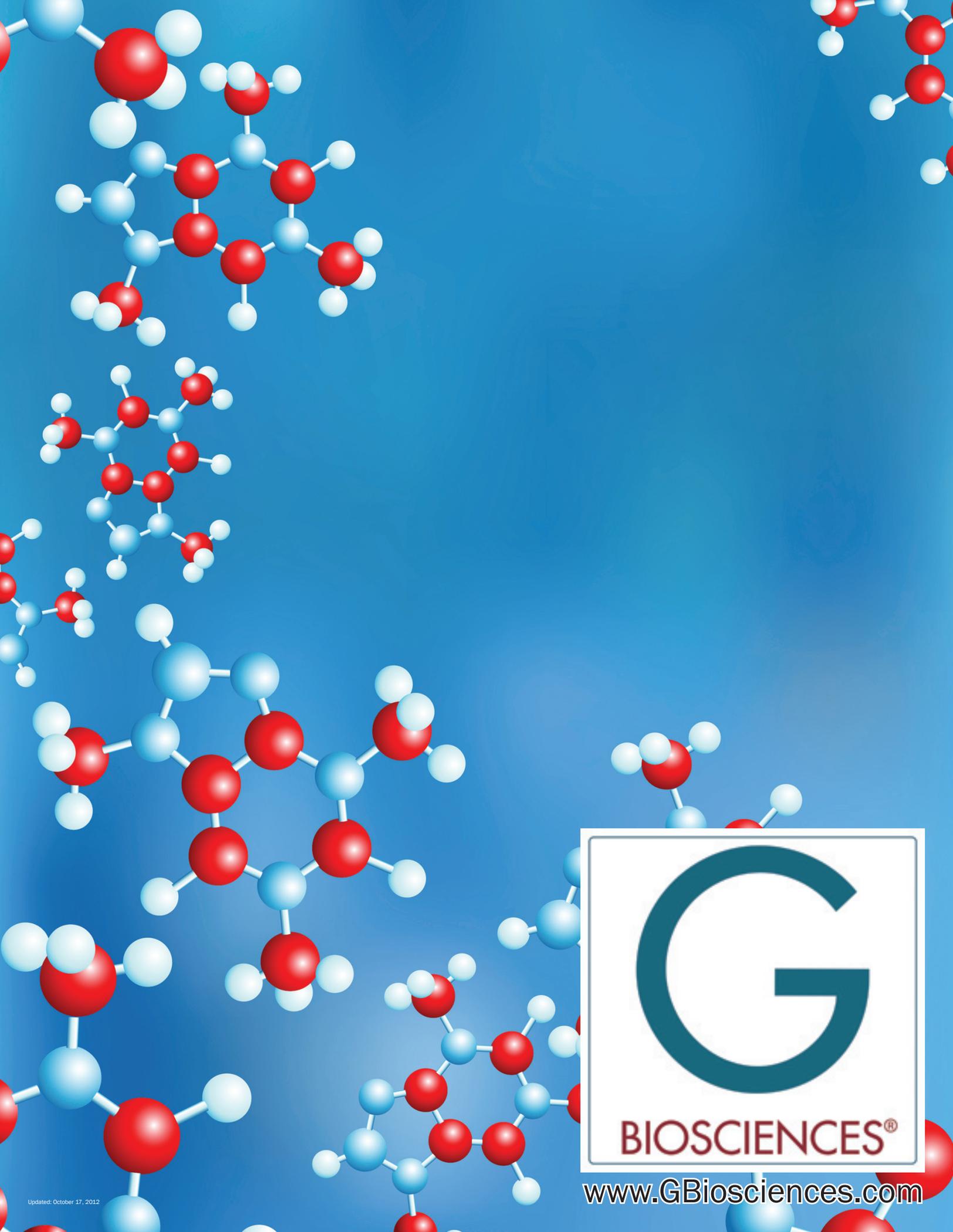
- Nickel resin
- Cobalt resin
- Copper resin
- Zinc Resin
- Glutathione Resin
- Streptavidin Resin
- Calmodulin Resin

- Carrier Proteins
  - Peptide Coupling
  - Protein A or G Resin
  - Activated Resins
  - Pearl Resin
  - Thiophilic Resin
  - Ficin
  - Pepsin
  - Papain

- BSA
- KLH
- HyperCarrier

- Assays
- Substrates
- Inhibitors

- Tissue
- Blood
- Plant
- Yeast
- Bacteria
- Fungi
- Mouse Tail



[www.GBiosciences.com](http://www.GBiosciences.com)