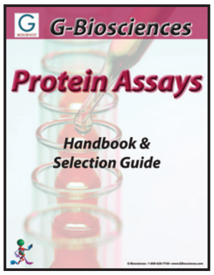
G-Biosciences

Protein Cross-Linkers

Handbook & Selection Guide



- Protein Estimation Assays
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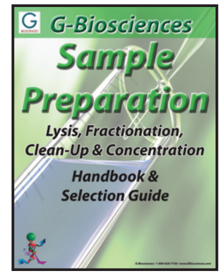

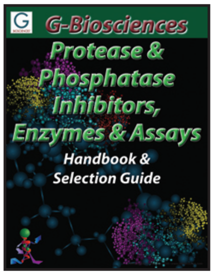

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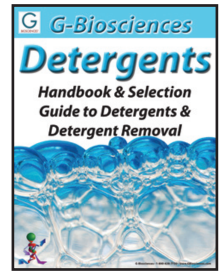

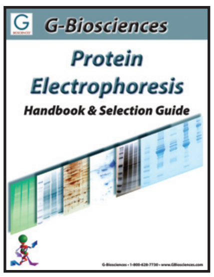

- Gel Preparation Chemicals
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- 1-Hour Western System
- Transfer Buffers & Membranes
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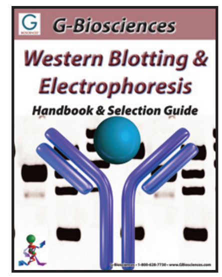

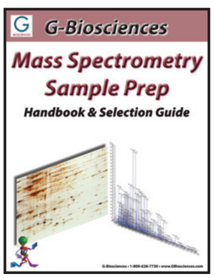

- Protein Sample Preparation
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- 6X His Protein Purification Kits
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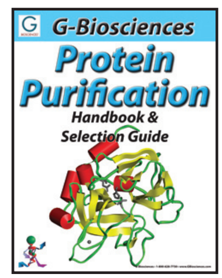

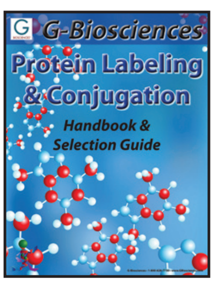

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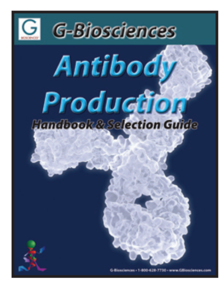

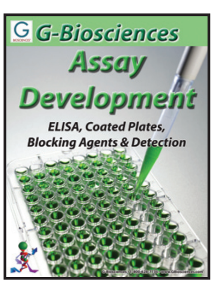

- Coated Plates
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- Homobifunctional
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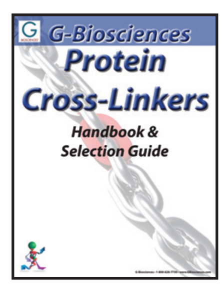
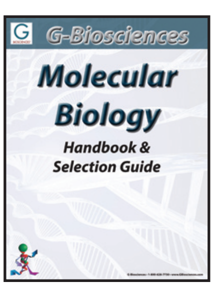
- Optimizer Systems
- Cross-Linking Systems





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

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- Protease Assays
- Phosphatase Assays
- Peroxide Assay
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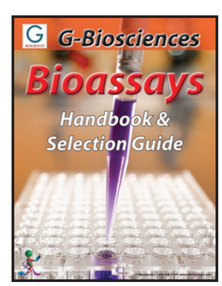

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Updated: May 11, 2012

Introduction

Cross-linking agents contain at least two reactive groups that are reactive towards numerous groups, including sulfhydryls, amines and carbohydrates, and create chemical covalent bonds between two or more molecules. Functional groups that can be targeted with cross-linking agents are primary amines, carboxyls, sulfhydryls, carbohydrates and carboxylic acids. Protein molecules have many of these functional groups and therefore proteins and peptides can be readily conjugated using cross-linking agents. Cross-linking agents are used to study protein structure and function, to anchor proteins to solid supports, preparation of immunogens, immunotoxins, and other conjugated protein reagents.

CROSS-LINKING APPLICATIONS

Structural & Functional Studies

Cross-linking agents are used to study the structure and composition of protein molecules. Cross-linking can answer questions about the subunit composition of a protein, protein conformations, various protein folding patterns, and so forth. Cross-linkers can be used to stabilize protein conformational changes.

Use of heterobifunctional cross-linkers may identify specific amino acids and their location within the molecules. Cleavable cross-linkers may be used to identify subunit structures. After conjugation, the protein is subject to two-dimensional electrophoresis. When subunits are coupled with a cross-linker, the protein molecules migrate as a single protein band. After cleaving the cross-linked protein in second dimension, the single band will resolve into constituent subunits.

Cross-linkers with short-to-medium spacer arms are suitable for intramolecular cross-linking, while cross-linkers with long spacer arms are suitable for intermolecular cross-linking. Protein and reagent concentration may also effect intermolecular cross-linking as high concentrations of homobifunctional cross-linkers and dilute protein solution favors formation of intramolecular cross-linking.

Protein & Receptor Interactions

Protein cross-linkers can be used to establish protein-to-protein association and ligand-receptor interactions. Since the distance between two potential molecules are known, it is often preferable to use a panel of similar cross-linkers with different spacer arm lengths. Both cleavable and non-cleavable cross-linkers can be used. Similarly, homo and heterobifunctional cross-linkers can be used.

For Immunological Tools

Antibody production routinely couples haptens, polypeptides and peptides to carrier proteins using a wide variety of cross-linkers. The choice of a cross-linker is dictated by the functional groups present on the hapten and carrier proteins, with the amine groups being the preferred group on carrier proteins. Peptides are often synthesized with terminal cysteines that are conjugated to carrier proteins using sulfhydryl-amine reactive heterobifunctional cross-linkers. Carbodiimides are also a popular cross-linker for producing protein-peptide conjugates, since both proteins and peptides usually contain several carboxyls and amines.

Cell Membrane Structural Studies

Cross-linkers are useful for studying structure and function of membrane proteins. Cross-linking will locate various proteins on both sides of a membrane. Suitable cross-linkers for membrane study can penetrate the lipid bilayer environment. Imidoester cross-linkers are water soluble but they are able to penetrate a membrane. Water soluble cross-linkers are suitable for establishing the location of molecules on the outer layer of a membrane. Any combination of hydrophobic and hydrophilic cross-linkers may be used for a complete picture. Sulfhydryl reactive cross-linkers are useful for targeting the molecules with cysteine.

Cell Surface Studies

Cross-linkers have been successfully used for identifying receptors on cell surfaces. Membrane impermeable cross-linkers, when used carefully and under controlled conditions, only react with molecules on the cell surface. The sulfo-NHS-esters are membrane impermeable and are a good choice for cross-linking proteins on cell surfaces. For determination of whether a protein is located on the cell surface, cell membrane preparation is conjugated with a known protein or a radioactive tag using a membrane impermeable cross-linker. After conjugation, the cell membrane preparation is analyzed by SDS-polyacrylamide gel electrophoresis.

Solid-Phase Immobilization

A wide variety of affinity supports are prepared by cross-linking proteins, peptides, and other molecules to a solid support. Nitrocellulose membrane, polystyrene, glass and agarose are among the most popular supports. Some of these supports can be activated for coupling, and others are available with functional groups that can be coupled with proteins or other molecules. Spacers can be attached to overcome steric hindrance. Useful spacer arms are diaminodipropylamine (DADPA), ethylenediamine, hexanediamine, and 6-amino-capronic. Amino acids and peptide can also be used.

Preparation of Immunotoxins

Toxic agents can be coupled to specific antibodies and used as a means to deliver toxins to a specific site within a cell. Immunotoxins are useful for killing specific cells such as tumor cells. These antibodies are often specific to tumor-associated antigens. For optimal immunotoxin effects, the immunotoxins often need to be released upon delivery. Cleavable disulfide-containing cross-linkers have been found to be more useful than non-cleavable cross-linkers. Cells are able to cleave the disulfide bond and release the toxin irreversibly.

Protein-Protein Conjugation

Protein-protein conjugation is one of the most common applications of a cross-linker. Protein-protein cross-linking is used for the preparation of enzyme coupled antibody probes; protein coupling to chromospheres, fluorophores, and other molecules. Enzymes such as alkaline phosphatase and peroxidase coupled to primary and secondary antibodies are among the most widely used protein-protein conjugation.

One of the widely used methods of protein-protein conjugation is through carbohydrate moieties, called reductive alkylation or amination. Carbohydrate moieties can be oxidized and then coupled with primary amines on enzymes. These conjugations are superior to glutaraldehyde conjugations, which produce high background.

If two proteins contain sulfhydryls, homobifunctional sulfhydryl cross-linkers may be used to couple them. Other homobifunctional cross-linkers such as NHS-esters or imidoester may also be used. Homobifunctional cross-linkers have the potential of producing self-conjugation or polymerization. Heterobifunctional cross-linkers, on the other hand, do not pose the risk of self-conjugation and hence are the best choice for antibody-enzyme and other protein-protein conjugations. For example, cross-linker SMCC or Sulfo-SMCC in a two-step reaction first conjugated with one protein. The second protein is thiolated with SATA and then conjugated with the first protein.

Protein to DNA/RNA Cross-Linking

DNA probes are synthesized with amine or thiol groups attached to specific bases, which act as target reactive sites for cross-linking.

Reactive Group Transfer

Cross-linkers may be used to modify target groups and add space for subsequent coupling reactions. For example, amine activated support can be converted to sulfhydryl with NHS-ester maleimide.

Cross-Linkers: A wide selection of cross-linkers, their features, consideration for selection, and applications.

Optimizer Buffers™: Six Optimizer Buffers™ that have the ideal conditions for each Cross-Linker reagent. Simply exchange your buffer with the Optimizer Buffer™ and proceed with the reaction.

Tube-O-Reactor™: A complete dialysis reaction system that contains micro dialysis devices and dialysis cups.

SpinOUT™ Columns: 5µl to 4ml spin column, sample volumes for desalting and buffer exchange..

CROSS-LINKERS

Cross-linking agents can be divided into groups dependent on the number and similarity of the reactive groups:

- **Homobifunctional** have two reactive ends that are identical
- **Heterobifunctional** have two different reactive ends

Homobifunctional cross-linkers are used in one step reactions while the heterobifunctional cross-linkers are used in two step sequential reactions, where the least labile reactive end is reacted first. Homobifunctional cross-linking agents have the tendency to result in self-conjugation, polymerization, and intracellular cross-linking. On the other hand, heterobifunctional agents allow more controlled two step reactions, which minimizes undesirable intramolecular cross-reaction and polymerization.

The most widely used heterobifunctional cross-linking agents are used to couple proteins through amine and sulfhydryl groups. The least stable amine reactive NHS-esters couple first and, after removal of uncoupled reagent, the coupling to the sulfhydryl group proceeds. The sulfhydryl reactive groups are generally maleimides, pyridyl disulfides and α-haloacetyls. Other cross-linkers include carbodiimides, which link between carboxyl groups (-COOH) and primary amines (-NH_2). There are heterobifunctional cross-linkers with one photoreactive end. Photoreactive groups are used when no specific groups are available to react with as photoreactive groups react non-specifically upon exposure to UV light.

It is often desirable to minimize the degree of structural shift due to cross-linking reactions, and more so if the protein molecule is biologically active. Therefore, cross-linking is performed under mild buffer and pH conditions. Depending on the application, the degree of conjugation is also important and an optimal cross-linker to protein ratio must be maintained. The number of target groups on the outer surface of a protein is also important. If the exposed target groups are readily available for conjugation, a lower cross-linker to protein ratio can be used.

Cross-linkers are available with different spacer arm lengths. A cross-linker with a longer space arm may be used where two target groups are further apart. The availability of cross-linkers with different spacer arms allows optimization of cross-reaction efficiency. Cross-linkers with short space arms are suitable for intramolecular cross-linking. Cleavable cross-linkers are also available which extends the scope of protein analysis.

SELECTION OF PROTEIN CROSS-LINKERS

These features are taken into consideration when making selection of a cross-linker:

1. Reagent solubility
2. The nature of reactive groups
3. Homobifunctional or heterobifunctional
4. Photoreactive or thermoreactive groups
5. The length of the spacer arm
6. Conjugated product cleavable or not
7. Potential for further labeling
8. Reaction condition needed for conjugation

PRIMARY AMINE REACTIVE

Amines, lysine ε-amines and N-terminal α-amines, are the most abundant group in protein molecules and represent the most common target for cross-linking. 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.

IMIDOESTERS

Imidoesters react with primary amine targets and form amidine bonds. The reaction is rapid at alkaline pH and has a short half-life. As the pH becomes more alkaline, the reactivity increases; hence conjugation is more efficient at pH 10.0 than pH 8.0. Below pH 10.0, the reaction is likely to result in undesirable side reactions. However, the amidine formed is reversible at high pH.

Imidoesters are used for protein subunit studies, molecular interactions, and for immobilization of proteins to solid supports. Imidoesters have been used as a substitute for glutaraldehyde for tissue fixation. Imidoesters are membrane permeable and can be used for cross-linking within the confines of cell membranes to study membrane composition, structure and protein-protein interaction and other molecular interactions.

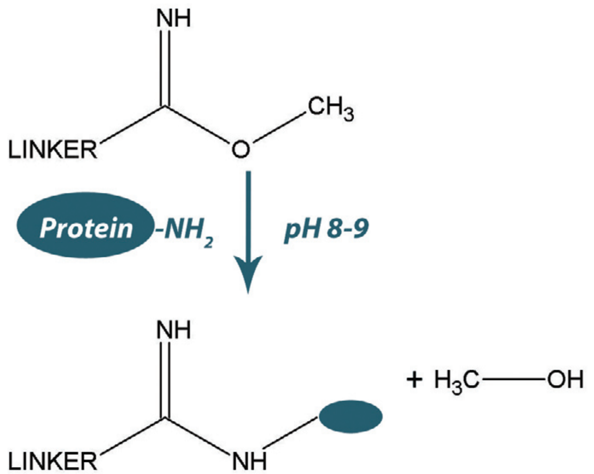


Figure 1: Coupling through imidoester groups.

N-HYDROXYSUCCINIMIDE-ESTERS (NHS-ESTERS)

NHS-Esters form stable products upon reaction with primary amines with relative efficiency at physiological pH. NHS-Esters react with α-amine groups present on the N-termini of proteins and α-amines on lysine residues to form an amide bond and release N-hydroxysuccinimide.

Hydrolysis of NHS-Ester competes with the primary amine reaction. Hydrolysis rate increases with increasing pH and occurs more readily in dilute protein solutions.

The most widely used cross-linkers that have an amine reactive group are the water insoluble, membrane permeable N-hydroxysuccinimide (NHS) esters or the water soluble, membrane impermeable N-hydroxysulfosuccinimide (sulfo-NHS) esters. Addition of a charged sulfonate (SO^{3-}) on the N-hydroxysuccinimide ring of the sulfo-NHS esters results in their solubility in water (~10mM), but 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.

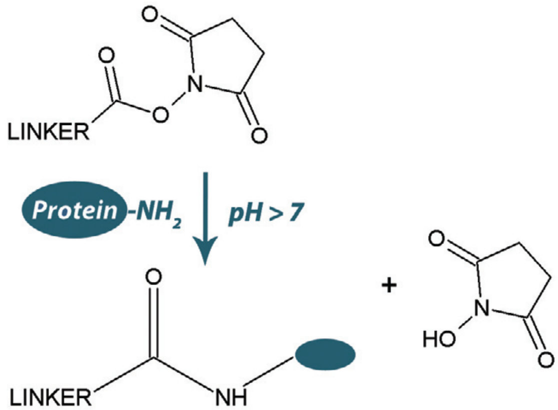


Figure 2: Coupling through NHS-ester groups.

For optimal amine coupling, use Optimizer Buffer™-I (Cat. No. BKC-04)

Water-insoluble NHS-Esters are first dissolved in organic solvents, such as DMSO or DMF, and then added to the aqueous reaction mixture. The reactions are typically performed with a solvent carryover of 0.5-10% in final volume in the aqueous reaction.

GENERAL PRECAUTIONS FOR AMINE CONJUGATION

Avoid buffers containing amines such as Tris or glycine.

AMINE REACTIVE		
Cat. No.	**Name**	**2nd Group**
BC01	BSOCOES	Amine
BC04	DSS	Amine
BC05	DST	Amine
BC06	sulfoDST	Amine
BC07	DSP	Amine
BC08	DTSSP	Amine
BC09	EGS	Amine
BC11	sulfoMBS	Sulfhydryl
BC12	GMBS	Sulfhydryl
BC13	sulfoGMBS	Sulfhydryl
BC14	EMCS	Sulfhydryl
BC16	sulfoEMCS	Sulfhydryl
BC17	SIAB	Sulfhydryl
BC19	SMCC	Sulfhydryl
BC20	SMPB	Sulfhydryl
BC21	sulfoSIAB	Sulfhydryl
BC22	sulfoSMCC	Sulfhydryl
BC23	sulfoSMPB	Sulfhydryl
BC24	EDC	Carboxyl
BC25	Mal-PEG-NHS	Sulfhydryl
BC27	ANB-NOS	Photoreactive
BC29	NHS-ASA	Photoreactive
BC34	sulfoHSAB	Photoreactive
BC35	sulfoSAED	Photoreactive
BC36	sulfoSAND	Photoreactive
BC37	sulfoSANPAH	Photoreactive
BC38	sulfoSADP	Photoreactive
BC39	sulfoSASD	Photoreactive

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 either the reduction of disulfides (-S-S-) with reducing agents such as mercaptoethylamine; or by modifying lysine ε-amines with Traut's reagent or SATA. If disulfide bond reduction is used, then excess reducing agent must be removed before reaction with sulfhydryl reactive reagents. In addition, a metal chelating agent (EDTA) as an anti-oxidant reduces the chances of reoxidation of sulfhydryls to disulfides. There are three different reactions employed to cross-link to sulfhydryl residues and involve either maleimides, haloacetyls or pyridylthiol groups.

MALEIMIDES

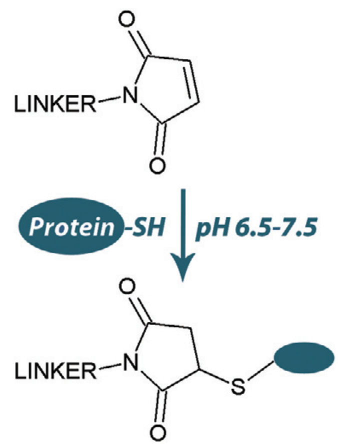


Figure 3: Coupling through maleimide groups.

The maleimide group is more specific for sulfhydryl residues than the other reactive groups. At pH 7 maleimide groups are 1000 fold more reactive toward free sulfhydryls than amines. At pH>8.5, maleimide groups favor primary amines. Conjugation is carried out at pH 6.5-7.5 for minimizing the reaction toward primary amines. At higher pH, >8.00, hydrolysis of maleimide to maleamic acid also increases, which can compete with thiol modification.

Optimizer Buffer™-III (Cat. No. BKC-06) provides ideal conditions for maleimide coupling reactions

HALOACETYLS

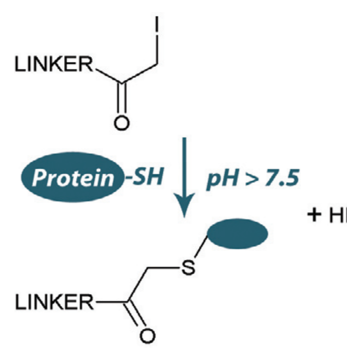


Figure 4: Coupling through iodoacetyl groups.

The most commonly used haloacetyls contain the iodoacetyl groups that react with sulfhydryl groups at physiological pH to form thioether bonds. Using slight excess of iodoacetyl group at ~pH 8.2 ensures selective reaction with sulfhydryl groups. Iodoacetyl reaction should be performed in dark to limit the formation of free iodine, which has the potential to react with tyrosines, tryptophans, and histidines.

For optimal iodoacetyl conjugation, we recommend Optimizer Buffer™-II (Cat. No. BKC-05)

PYRIDYL DISULFIDES

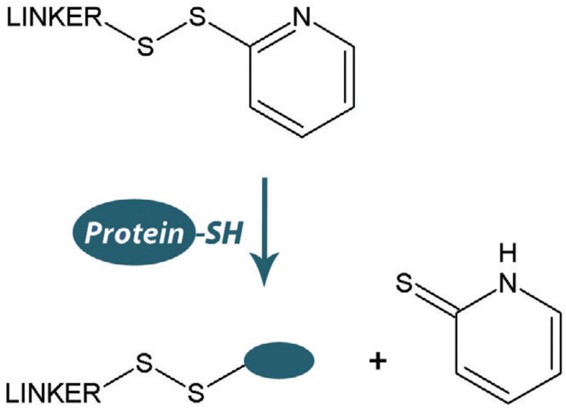


Figure 5: Pyridyldithiol group coupling.

Pyridyl disulfides, also known as pyridyldithiols, react with free sulfhydryls 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 the cross-linking agent 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 (Cat. No. BKC-06) provides the optimized conditions

GENERAL PRECAUTIONS FOR SULFHYDRYL REACTIVE REAGENTS:

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

SULFHYDRYL REACTIVE		
Cat. No.	**Name**	**2nd Group**
BC03	DPDPB	Sulfhydryl
BC11	MBS	Amine
BC12	sulfoMBS	Amine
BC13	GMBS	Amine
BC14	sulfoGMBS	Amine
BC15	EMCH	Carbohydrate
BC16	EMCS	Amine
BC17	sulfoEMCS	Amine
BC18	PMPI	Hydroxyl
BC19	SIAB	Amine
BC20	SMCC	Amine
BC21	SMPB	Amine
BC22	sulfoSIAB	Amine
BC23	sulfoSMCC	Amine
BC24	sulfoSMPB	Amine
BC27	Mal-PEG-NHS	Amine
BC32	APDP	Photoreactive

CARBOHYDRATE REACTIVE

Some cross-linking reagents do not bind directly to the protein itself but conjugate to the carbohydrate residues of glycoproteins. Carbohydrate reactive cross-linking reagents contain hydrazides (-NH-NH_2) 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.

For reaction with glycoproteins, the first step is to generate carbonyl groups that react with hydrazide, under mild oxidizing conditions with sodium periodate ($NaIO_4$). 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 (Cat. No. BKC-08) is recommended

GENERAL PRECAUTIONS FOR CARBOHYDRATE REACTIVE REAGENTS:

Each glycoprotein has an optimal pH for oxidation and optimal pH for the hydrazide reaction. Periodate oxidation is dependent on temperature and 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.

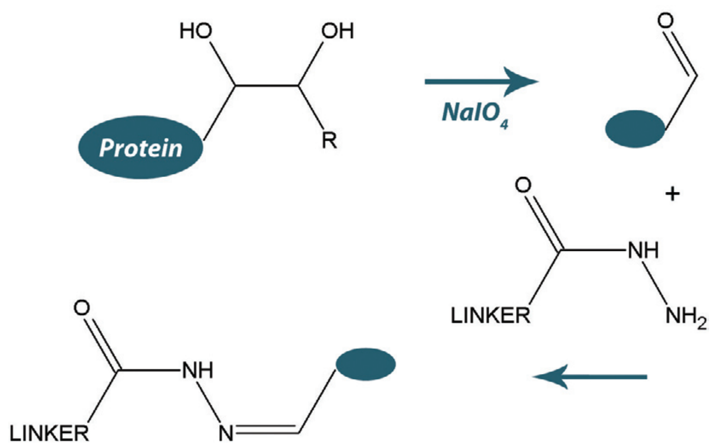


Figure 6: Coupling to Carbohydrates.

CARBOHYDRATE REACTIVE		
Cat. No.	**Name**	**2nd Group**
BC15	EMCH	Sulfhydryl
BC28	ABH	Photoreactive

CARBOXYL REACTIVE

Cross-linking to carboxyl groups is mediated by a water-soluble carbodiimide. Carbodiimides effect conjugation of carboxyl to primary amines or hydrazides and result in formation of amide or hydrazone bonds. The conjugation is performed between pH 4.5 to 7.5; however, reaction conditions of pH 4.5-5.0 are generally recommended. The reaction takes only a few minutes to complete. The carboxyl termini of proteins, glutamic acid and aspartic acid side chain are targets. Since there is an abundance of both carboxyl and primary amine groups in protein, in the presence of excess of carbodiimides, polymerization may occur. Since there is no spacer between the reacting groups, carbodiimides are called zero spacer arm cross-linkers and the resulting bond is the same as a peptide bond.

Carbodiimides react and activate the carboxylic acid groups to form an active intermediate (O-acylisourea). This intermediate reacts with a primary amine to form an amide derivative.

The O-acylisourea intermediate is unstable in aqueous medium and the failure to react with amine results in hydrolysis and formation of an N-unsubstituted urea and regeneration of the carboxylic groups.

The intermediate O-acylisourea can be stabilized with NHS-esters. When NHS-esters are combined in the reaction, carbodiimides couple NHS to carboxyl, resulting in an NHS-activated molecule that is amine-reactive. In the reaction mixture, both O-acylisourea intermediates and NHS-activated molecules compete for amine targets. In aqueous medium, NHS-esters have a longer half-life than O-acylisourea with the half-life of NHS-ester measured in one to several hours and even days (depending on temperature and pH), where as O-acylisourea has a half-life measured in seconds in acidic to neutral pH. Addition of NHS-esters is necessary when the protein concentration is very low.

The hydrolysis of carbodiimide is a competing reaction and is dependent on temperature, pH, and buffer composition. Tris, glycine, and acetate buffers are not recommended. Phosphate buffers reduce coupling efficiency, which can be compensated by increasing the concentration of carbodiimides.

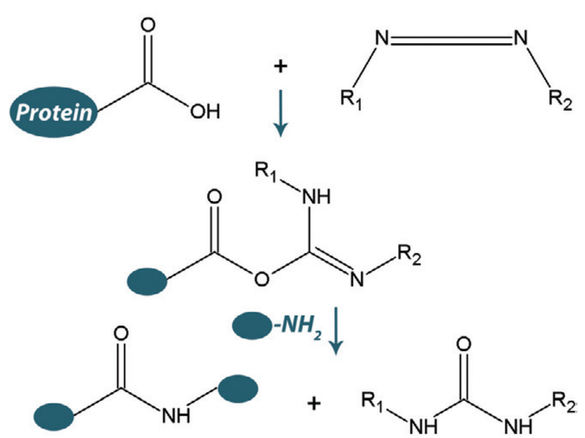


Figure 7: Coupling with Carboxyl.

Optimizer Buffer™-IV (Cat. No. BKC-07) provides the ideal buffer for EDC and other carbodiimides

GENERAL PRECAUTIONS FOR CARBOXYL REACTIVE REAGENTS:

EDC may cross-link protein, decreasing EDC minimizes polymerization.

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.

CARBOXYL REACTIVE		
Cat. No.	**Name**	**2nd Group**
BC25	EDC	Amine
BC28	ABH	Photoreactive

PHOTOREACTIVE

On exposure to ultraviolet light, photoreactive agents 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 cross-linking reagents for the coupling of proteins, peptides, nucleic acids, and other molecules.

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 (Cat. No. BKC-04) provides excellent buffer conditions

GENERAL PRECAUTIONS FOR PHOTOREACTIVE REAGENTS:

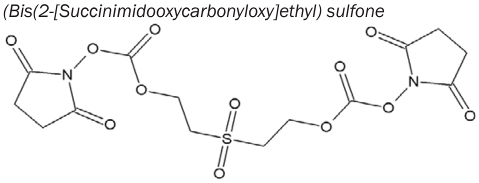
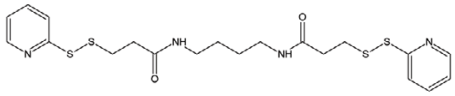
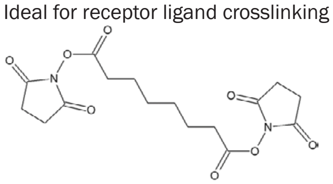
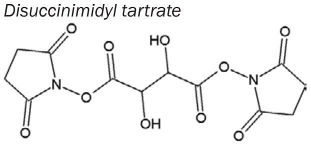
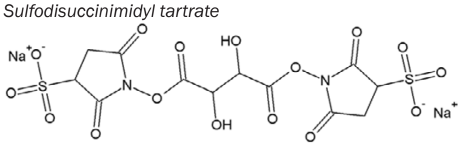
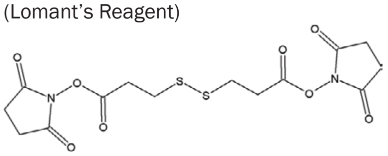
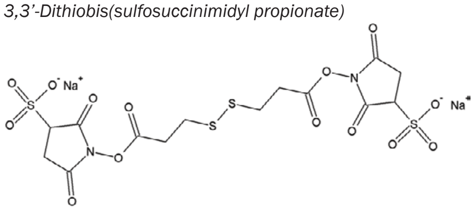
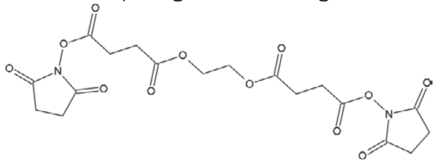
Avoid acidic and reducing agents since they inactivate aryl azide groups.

PHOTOREACTIVE		
Cat. No.	**Name**	**2nd Group**
BC28	ABH	Carbohydrate
BC29	ANB-NOS	Amine
BC30	APG	Arginine
BC32	APDP	Sulfhydryl
BC33	BASED	-
BC34	NHS-ASA	Amine
BC35	sulfoHSAB	Amine
BC36	sulfoSAED	Amine
BC37	sulfoSAND	Amine
BC38	sulfoSANPAH	Amine
BC39	sulfoSADP	Amine
BC40	sulfoSASD	Amine

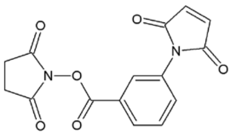
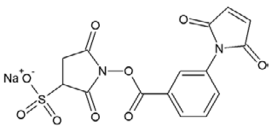
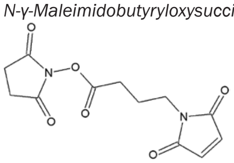
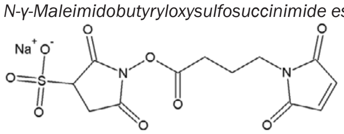
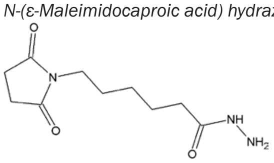
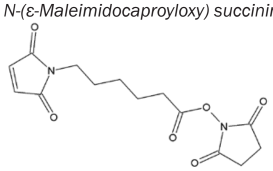
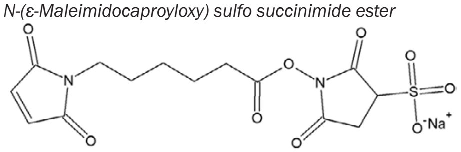
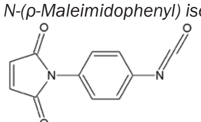
Cross-linker Selection Guide & Ordering Information

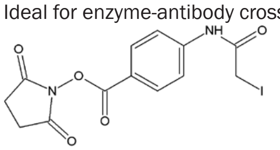
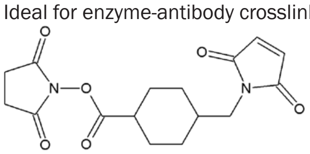
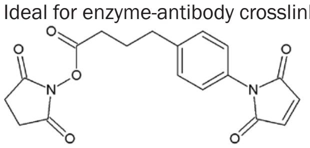
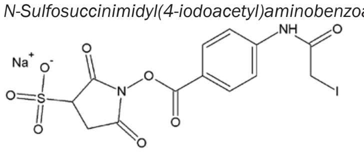
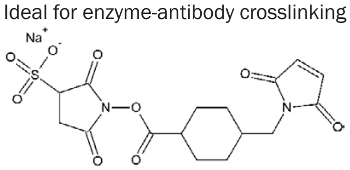
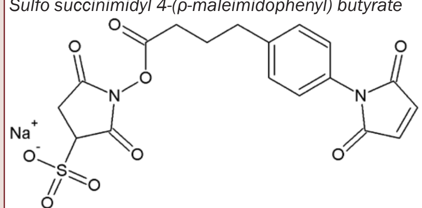
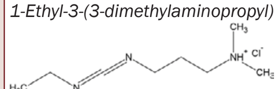
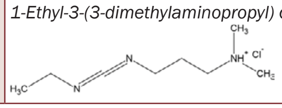
To select a cross-linking reagent several factors need to be considered:

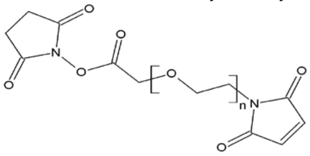
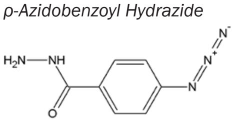
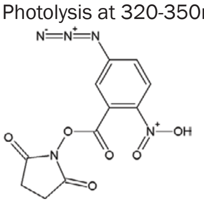
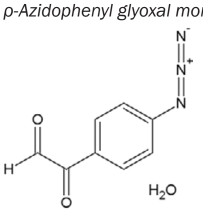
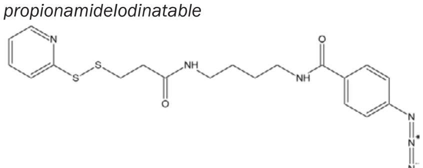
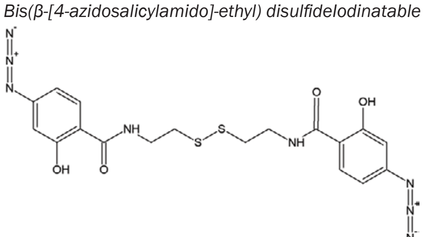
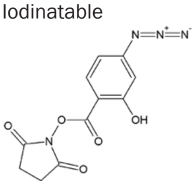
- **Reactive Toward:** Determines the target residues to be cross-linked, select a reagent that does not interfere with protein's function.
- **Membrane Permeability:** For cell surface labeling, select non-membrane permeable reagents.
- **Cleavable:** For easy release of cross-linked proteins from solid supports or for further downstream applications.
- **Reversible:** An alternative to cleavable reagents are reversible reagents. For example, ANB-NOS is released by photolysis.
- **Steric Hinderance:** Bulky groups around the binding site may require reagents with longer spacer arms.

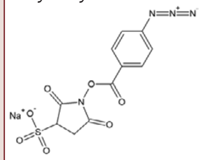
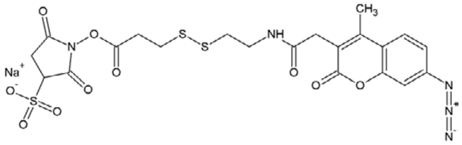
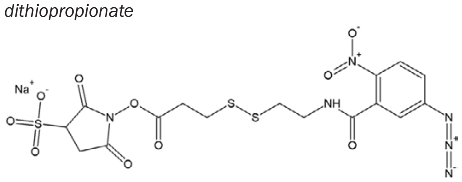
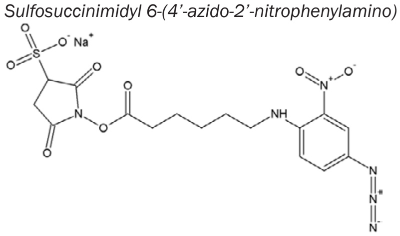
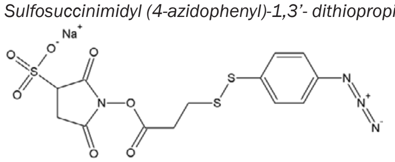
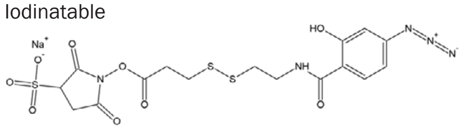
Cat. No.	Cross Linking Reagent	Quantity Supplied	Molecular Weight	Spacer Arm (Å)	Reactive Toward	Membrane Permeable	Water Soluble	Cleavable/ Reversible
HOMOBIFUNCTIONAL CROSS LINKERS								
BC01	**BSOCOES** *(Bis(2-[Succinimidooxycarbonyloxy]ethyl) sulfone* 	100mg	436.35	13	Primary Amines	YES	NO	Base
BC03	**DPDPB** *1,4-Di-(3'-[2'pyridyldithio]-propionamido) butane* 	100mg	482.71	19.9	Sulfhydryls	nd	NO	Reducing Agents (Thiols)
BC04	**DSS** *Disuccinimidyl suberate* Ideal for receptor ligand crosslinking 	1g	368.4	11.4	Primary Amines	YES	NO	NO
BC05	**DST** *Disuccinimidyl tartrate* 	1g	344.24	6.4	Primary Amines	YES	NO	Oxidizing Agents (Periodate)
BC06	**Sulfo DST** *Sulfodisuccinimidyl tartrate* 	100mg	548.32	6.4	Primary Amines	NO	YES	Oxidizing Agents (Periodate)
BC07	**DSP** *Dithiobis(succinimidyl propionate)* (Lomant's Reagent) 	1g	404.42	12	Primary Amines	YES	NO	Reducing Agents (Thiols)
BC08	**DTSSP** *3,3'-Dithiobis(sulfosuccinimidyl propionate)* 	100mg	608.51	12	Primary Amines	NO	YES	Reducing Agents (Thiols)
BC09	**EGS** *Ethylene glycol bis(succinimidyl succinate)* Ideal for receptor ligand crosslinking 	1g	456.36	16.1	Primary Amines	YES	NO	Hydroxylamine

HETEROBIFUNCTIONAL CROSS LINKERS

Cat. No.	Cross Linking Reagent	Quantity Supplied	Molecular Weight	Spacer Arm (Å)	Reactive Toward	Membrane Permeable	Water Soluble	Cleavable/ Reversible
BC11	**MBS** *m-Maleimidobenzoyl-N-hydroxysuccinimide ester* Ideal for hapten-carrier protein, toxin-antibody, enzyme-antibody crosslinking 	100mg	314.25	9.9	Primary Amine + Sulfhydryl	YES	NO	NO
BC12	**Sulfo MBS** *m-Maleimidobenzoyl-N-hydroxysulfosuccinimide ester* 	100mg	416.30	9.9	Primary Amine + Sulfhydryl	NO	YES	NO
BC13	**GMBS** *N-γ-Maleimidobutyryloxysuccinimide ester* 	100mg	280.23	6.8	Primary Amine + Sulfhydryl	YES	NO	NO
BC14	**Sulfo GMBS** *N-γ-Maleimidobutyryloxysulfosuccinimide ester* 	100mg	382.38	6.8	Primary Amine + Sulfhydryl	NO	YES	NO
BC15	**EMCH** *N-(ε-Maleimidocaproic acid) hydrazide* 	50mg	225.24	11.8	Sulfhydryl + Carbohydrate	nd	NO	NO
BC16	**EMCS** *N-(ε-Maleimidocaproyloxy) succinimide ester* 	100mg	308.29	9.4	Primary Amine + Sulfhydryl	YES	NO	NO
BC17	**Sulfo EMCS** *N-(ε-Maleimidocaproyloxy) sulfo succinimide ester* 	50mg	410.33	9.4	Primary Amine + Sulfhydryl	NO	YES	NO
BC18	**PMPI** *N-(p-Maleimidophenyl) isocyanate* 	50mg	214.18	8.7	Sulfhydryl + Hydroxyl	nd	NO	NO

Cat. No.	Cross Linking Reagent	Quantity Supplied	Molecular Weight	Spacer Arm (Å)	Reactive Toward	Membrane Permeable	Water Soluble	Cleavable/ Reversible
BC19	**SIAB** *N-Succinimidyl(4-iodoacetyl)aminobenzoate* Ideal for enzyme-antibody crosslinking 	100mg	402.14	10.6	Primary Amine + Sulfhydryl	YES	NO	NO
BC20	**SMCC** *Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate* Ideal for enzyme-antibody crosslinking 	100mg	334.32	11.6	Primary Amine + Sulfhydryl	YES	NO	NO
BC21	**SMPB** *Succinimidyl 4-(ρ-maleimidophenyl) butyrate* Ideal for enzyme-antibody crosslinking 	100mg	356.33	11.6	Primary Amine + Sulfhydryl	YES	NO	NO
BC22	**Sulfo SIAB** *N-Sulfosuccinimidyl(4-iodoacetyl)aminobenzoate* 	100mg	504.19	10.6	Primary Amine + Sulfhydryl	NO	YES	NO
BC23	**Sulfo SMCC** *Sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate* Ideal for enzyme-antibody crosslinking 	100mg	436.37	11.6	Primary Amine + Sulfhydryl	NO	YES	NO
786-082	**OneQuant™ Sulfo SMCC** *Sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate* Single use vials to minimize waste. No weighing required.	8 x5mg	436.37	11.6	Primary Amine + Sulfhydryl	NO	YES	NO
BC24	**Sulfo SMPB** *Sulfo succinimidyl 4-(ρ-maleimidophenyl) butyrate* 	100mg	458.38	14.5	Primary Amine + Sulfhydryl	NO	YES	NO
BC25-1	**EDC** *1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride* 	1g	191.70	0	Primary Amine + Carboxyl	NO	YES	NO
BC25-5	**EDC** *1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride* 	5g	191.70	0	Primary Amine + Carboxyl	NO	YES	NO

Cat. No.	Cross Linking Reagent	Quantity Supplied	Molecular Weight	Spacer Arm (Å)	Reactive Toward	Membrane Permeable	Water Soluble	Cleavable/ Reversible
BC27	**MAL-PEG-SCM** *Maleimide PEG succinimidyl carboxymethyl* 	100mg	~3400		Primary Amine + Sulfhydryl	nd	NO	NO
PHOTOREACTIVE CROSS LINKERS								
BC28	**ABH** *ρ-Azidobenzoyl Hydrazide* 	100mg	177.16	11.9	Carbohydrates	YES	NO	NO
BC29	**ANB-NOS** *N-5-Azido-2-nitrobenzyloxysuccinimide* Photolysis at 320-350nm 	100mg	305.20	7.7	Primary Amines	YES	NO	Photolysis at 320-350nm
BC30	**APG** *ρ-Azidophenyl glyoxal monohydrate* 	100mg	193.16	9.3	Arginines	YES	NO	NO
BC32	**APDP** *N-(4-[ρ-Azidosalicylamido]butyl)-3'-(2'-pyridyldithio) propionamideIodinatable* 	100mg	446.55	21	Sulfhydryl	YES	NO	Reducing Agents (Thiols)
BC33	**BASED** *Bis(β-[4-azidosalicylamido]-ethyl) disulfideIodinatable* 	100mg	474.52	21.3	Non Selective	YES	NO	Reducing Agents (Thiols)
BC34	**NHS-ASA** *N-Hydroxysuccinimidyl-4-azidosalicyclic acid* Iodinatable 	100mg	276.21	8.0	Primary Amines	YES	NO	NO

Cat. No.	Cross Linking Reagent	Quantity Supplied	Molecular Weight	Spacer Arm (Å)	Reactive Toward	Membrane Permeable	Water Soluble	Cleavable/ Reversible
BC35	**Sulfo HSAB** *N-Hydroxysulfosuccinimidyl-4-azidobenzoate* 	100mg	362.25	9.0	Primary Amines	NO	YES	NO
BC36	**Sulfo SAED** *Sulfosuccinimidyl 2-(7-amino-4-methylcoumarin-3-acetamido)ethyl-1,3-dithiopropionate* Fluorescent Label 	50mg	621.6	23.6	Primary Amines	NO	YES	Reducing Agents (Thiols)
BC37	**Sulfo SAND** *Sulfosuccinimidyl 2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate* 	100mg	570.51	18.5	Primary Amines	NO	YES	Reducing Agents (Thiol)
BC38	**Sulfo SANPAH** *Sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino) hexanoate* 	100mg	492.40	18.2	Primary Amines	NO	YES	NO
BC39	**Sulfo SADP** *Sulfosuccinimidyl (4-azidophenyl)-1,3'- dithiopropionate* 	100mg	454.44	13.9	Primary Amines	NO	YES	Reducing Agents (Thiol)
BC40	**Sulfo SASD** *Sulfosuccinimidyl-2-(ρ-azidosalicylamido)ethyl-1,3-dithiopropionate* Iodinatable 	100mg	541.51	18.9	Primary Amines	NO	YES	Reducing Agents (Thiol)

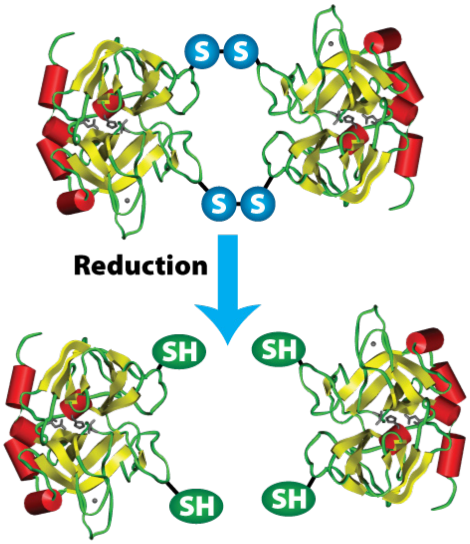


Figure 8: Protein Reduction scheme.

Protein-S-S-Reductant™

A water soluble, odorless, non-toxic and stable protein reductant. Protein-S-S-Reductant™ uses TCEP (Tris [2-carboxyethyl] phosphine), a popular alternative to β-mercaptoethanol and DTT (dithiothreitol). TCEP improves stability, increases effectiveness, and reduces proteins over a wider range of pH, including lower acidic pHs.

Protein-S-S-Reductant™ completely reduces stable disulfide bonds in less than 5 minutes at room temperature and is compatible with the protein alkylation reactions.

Protein-S-S-Reductant™ is a ready-to-use solution that is at neutral pH and stabilized for long-term storage (1 year).

Simply supplement Protein-S-S-Reductant™ in place of DTT or β-mercaptoethanol and boil the sample.

FEATURES
- Ready-to-use solution, odorless, stable and non-toxic
- Neutral protein reduction solution
- Compatible with the alkylation reaction
- Works over a wide range of pH, including lower acidic pHs

APPLICATIONS
- Reduction of protein disulfide bonds
- Reduction for protein electrophoresis and other applications

Cat. No.	Description	Size
786-25PR	Protein-S-S-Reductant™	200 Preps

Immobilized Reductant

Immobilized Reductant is an immobilized form of cysteine thiolactone covalently coupled to agarose beads that allows for a fast and reliable reduction of disulfide bridges in protein and peptide solutions.

Reducing agents are used in the reduction of disulfide bonds of proteins and peptides. Often it is necessary to remove the reducing agents from the protein/peptide solutions to prevent them interfering with subsequent procedures. For small proteins and particularly peptides it is almost impossible to remove the reducing agent from the protein/peptide using the standard practice of gel filtration, as the small proteins and peptides elute with the reducing agents. Immobilized Reductant is perfect for the reduction of small proteins and peptides as the reducing agent remains securely bound to the resin.

The Immobilized Reductant is supplied as 2ml resin in a column that can regenerated and reused for a total of five uses.

FEATURES
- No contamination of sample with soluble reducing agents, i.e. DTT, TCEP, Mercaptoethanol
- No gel filtration or other clean up step required to remove reductant
- Regenerate column up to four times
- Reduce both peptide and protein solutions

APPLICATIONS
- Reduction of protein and peptide solutions

Cat. No.	Description	Size
786-148	Immobilized Reductant	2ml resin

TCEP

Tris [2-carboxyethyl] phosphine hydrochloride (TCEP.HCl) for researchers who wish to prepare their own solutions. Available in convenient 1gm quantities.

CITED REFERENCES

Jamaluddin, M et al (2010) J. Virol. 84:9533

Cat. No.	Description	Size
786-030	TCEP	1g

Dithiothreitol (DTT)

A common reducing agent used for the cleavage of disulfide bonds. DTT is supplied in bulk 5 gram quantities.

OneQuant™ DTT are single aliquots of DTT that eliminate the need for weighing; preventing loss of reagent and saving time. Add 90µl water to a single tube to generate a 0.5M DTT solution. Supplied with 40 individual tubes.

Cat. No.	Description	Size
BC99	DTT	5g
786-077	OneQuant™ DTT [0.5M]	40 vials

ß-Mercaptoethanol

A popular reducing agent, is offered in 100ml bottles.

Cat. No.	Description	Size
BC98	β-mercaptoethanol	100ml

Ellman's Reagent

5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB)
For quantifying free sulfhydryl groups

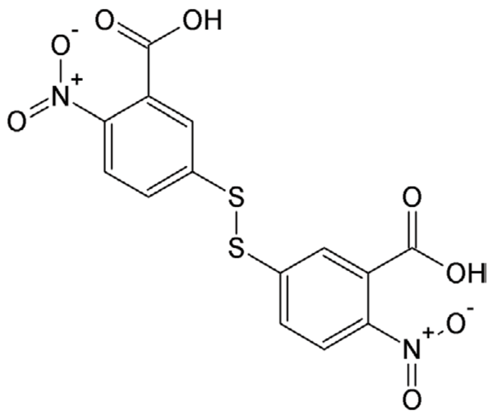


Figure 9: Structure of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB).

A versatile, water-soluble compound for quantifying free sulfhydryl groups in solution. It reacts with a free sulfhydryl group to yield a mixed disulfide and 2-nitro-5-thiobenzoic acid (NTB), a measurable yellow colored product at 412nm.

Ellman's reagent is very useful as a free sulfhydryl assay reagent due to its high specificity for -SH groups at neutral pH, high molar extinction coefficient and short reaction time.

APPLICATIONS

- Quick and simple quantification of free sulfhydryl residues

Cat. No.	Description	Size
BC87	Ellman's Reagent	5g

Reaction Accessories

Protein cross-linking and modification reactions often required carefully controlled reaction conditions as some buffers will interfere with coupling and modification reactions. For example, Tris buffers will interfere with amine coupling reactions.

G-Biosciences offers a selection of optimized buffers designed for specific reactions.

G-Biosciences also offers a reactor system, Tube-O-Reactor™, that is ideal for protein cross-linking and modification reactions.

Optimizer Buffer™

For optimal cross-linking & modification reaction conditions

The conjugation and modification reactions used to cross-link proteins or couple labels to proteins, such as biotin, enzymes, and fluorescent dyes, require certain conditions, including pH and chemical composition, for optimal conjugation. Many common buffers routinely used in laboratories have an inhibitory effect on conjugation reactions, for example Tris buffers inhibit coupling to amines.

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

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

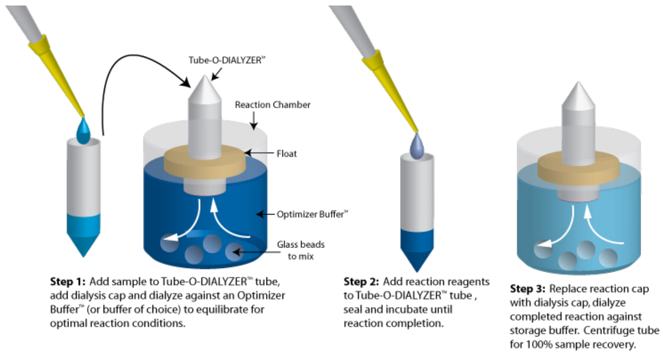


Figure 10: Tube-O-Reactor™ system.

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

Solvents

Anydrous DMSO and DMF are offered for the solubilization of the water insoluble cross-linkers and modification reagents.

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

Protein cross-linking and modification experiments often require the use of additional systems to remove the cross-linkers, chemicals and other reaction by-products.

DIALYSIS SYSTEMS

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

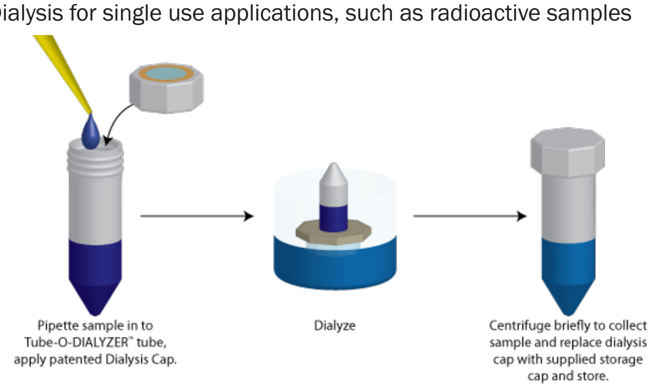


Figure 11: A summary of the Tube-O-DIALYZER™ system.

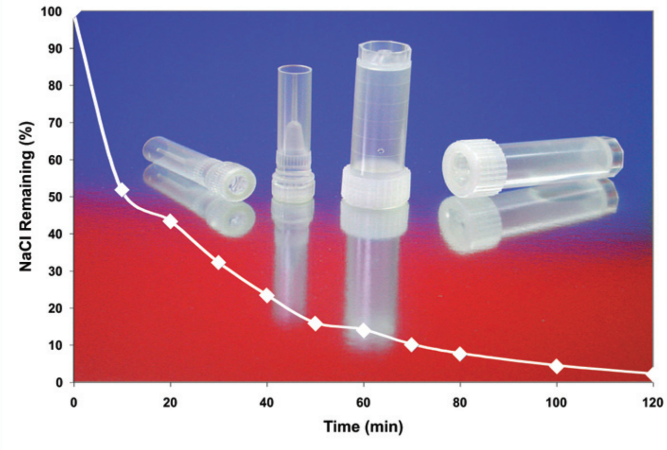


Figure 12: Tube-O-DIALYZER™ micro (8K MWCO) Dialysis Rate. 100µl 5M sodium chloride was dialyzed against 1 liter deionized water. 50% sodium chloride is removed in the first 10 minutes.

CITED REFERENCES

Bansal, P., et al (2009) Biol. Reprod. 81: 7
Ehmsen, K., et al (2008) Nucleic Acids Res. 36: 2182
Murdoch, A., et al (2007) Stem Cells. 25: 2786
Palmer, C., et al (2008) PLoS ONE 3: e2633
Holt, T. et al (2008) Electrophoresis. 26: 4486
Baechle, D. et al (2006) J. Biol Chem. 281: 5406
Finlay, W. et al (2005) Clinical and Experimental Allergy. 35 (8): 1040
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
786-611	Tube-O-DIALYZER™, Micro, 4k MWCO	20
786-612	Tube-O-DIALYZER™, Micro, 8k MWCO	20
786-613	Tube-O-DIALYZER™, Micro, 15k MWCO	20
786-614	Tube-O-DIALYZER™, Micro, 50k MWCO	20
786-615	Tube-O-DIALYZER™, Medi, 1k MWCO	20
786-616	Tube-O-DIALYZER™, Medi, 4k MWCO	20
786-617	Tube-O-DIALYZER™, Medi, 8k MWCO	20
786-618	Tube-O-DIALYZER™, Medi, 15k MWCO	20
786-619	Tube-O-DIALYZER™, Medi, 50k MWCO	20
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: $(Vi/Vo)^{\#C}$, where Vi is the volume inside a dialysis bag; Vo 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.

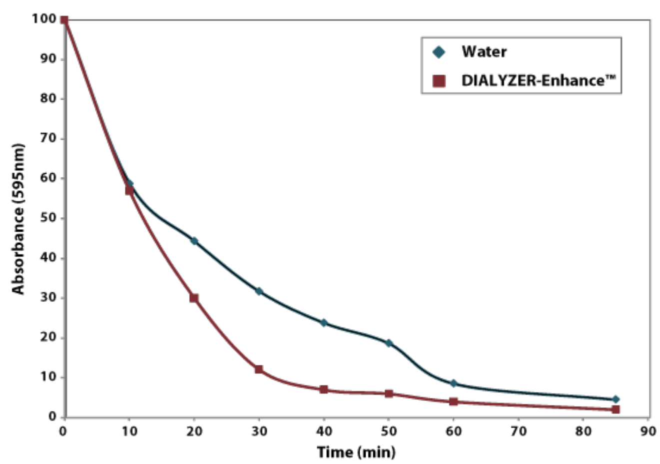


Figure 13: DIALYZER-Enhance™ reduces dialysis times. 0.5ml 5M NaCl was placed in a 8,000 MWCO Tube-O-DIALYZER™ dialyzed against 20ml water or 20ml water supplemented with DIALYZER-Enhance™.

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 diaysis 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: 786-141F is designed for dialysis in Tube-O-Tanks or a beaker and 786-149 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

DESALTING & BUFFER EXCHANGE

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 (ml)	Sample Load (ml)
786-703	SpinOUT™ GT-600, 0.1ml	25 columns	0.1	0.005-0.02
786-170	SpinOUT™ GT-600, 1ml	10 columns	1	0.05-0.1
786-171	SpinOUT™ GT-600, 3ml	10 columns	3	0.1-0.5
786-704	SpinOUT™ GT-600, 5ml	5 columns	5	0.5-2
786-705	SpinOUT™ GT-600, 10ml	5 columns	10	0.5-4
786-706	SpinOUT™ GT-1200, 0.1ml	25 columns	0.1	0.005-0.02
786-172	SpinOUT™ GT-1200, 1ml	10 columns	1	0.05-0.1
786-173	SpinOUT™ GT-1200, 3ml	10 columns	3	0.1-0.5
786-707	SpinOUT™ GT-1200, 5ml	5 columns	5	0.5-2
786-708	SpinOUT™ GT-1200, 10ml	5 columns	10	0.5-4

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

G-Biosciences Product Line Overview

Protein Research

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

BioAssays

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

Molecular Biology

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

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