G-Biosciences

Double-Do™

Protein Cross-Linkers

Handbook & Selection Guide

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.

G-Biosciences offers a variety of ***Double Do***™ cross-linking agents, reaction specific buffers, and accessories for performing and facilitating cross-linking applications. These accessory tools simplify cross-linking reactions and will enable researchers to achieve highly efficient cross-linking reactions with minimal effort in reaction optimization, set-up, and procedure development.

Cross-Linking Products

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

Optimizer Buffers™: G-Biosciences' researchers have carried out extensive studies into these reactions and have designed six Optimizer Buffers™ that have the ideal conditions for each Double Do™ 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.

Double Do™ *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

Cross-Linking Applications

Protein 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 Interaction and Receptor Studies

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.

Conjugation 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 as spacers.

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 in the cross-linker 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 tend to 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 reactions.

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.

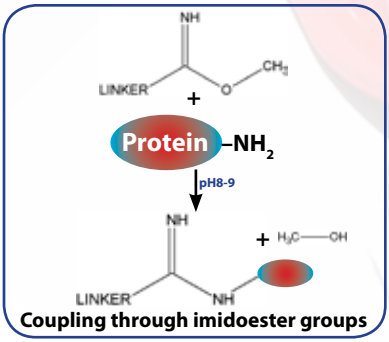
Primary Amine Reactive Cross-Linkers

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 pH10.0 than pH8.0. Below pH10.0, 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.



Coupling through imidoester groups

AMINE REACTIVE					
Cat. #	**Name**	**2nd Group**	**Cat. #**	**Name**	**2nd Group**
BC01	BSOCOES	Amine	BC20	SMCC	Sulfhydryl
BC04	DSS	Amine	BC21	SMPB	Sulfhydryl
BC05	DST	Amine	BC22	sulfoSIAB	Sulfhydryl
BC06	sulfoDST	Amine	BC23	sulfoSMCC	Sulfhydryl
BC07	DSP	Amine	BC24	sulfoSMPB	Sulfhydryl
BC08	DTSSP	Amine	BC25	EDC	Carboxyl
BC09	EGS	Amine	BC27	Mal-PEG-NHS	Sulfhydryl
BC10	sulfoEGS	Amine	BC29	ANB-NOS	Photoreactive
BC11	MBS	Sulfhydryl	BC34	NHS-ASA	Photoreactive
BC12	sulfoMBS	Sulfhydryl	BC35	sulfoHSAB	Photoreactive
BC13	GMBS	Sulfhydryl	BC36	sulfoSAED	Photoreactive
BC14	sulfoGMBS	Sulfhydryl	BC37	sulfoSAND	Photoreactive
BC16	EMCS	Sulfhydryl	BC38	sulfoSANPAH	Photoreactive
BC17	sulfoEMCS	Sulfhydryl	BC39	sulfoSADP	Photoreactive
BC19	SIAB	Sulfhydryl	BC40	sulfoSASD	Photoreactive

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.

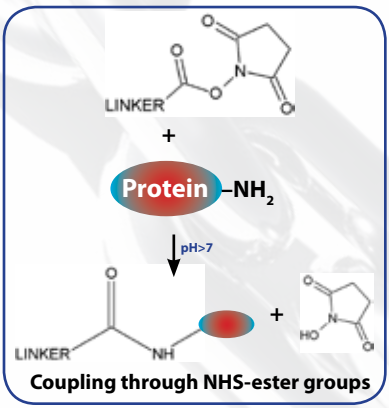
The reaction of the NHS and sulfo-NHS esters with amines are virtually identical leading to the formation of an amide bond and release of NHS or sulfo-NHS.

For optimal amine coupling, use Optimizer Buffer™-I.

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.



Coupling through NHS-ester groups

Sulfhydryl Reactive Cross-Linkers

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

The maleimide group is more specific for sulfhydryl residues than the other reactive groups. At pH7 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 provides ideal conditions for maleimide coupling reactions.***

Haloacetyls

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 tyrosine, tryptophan, and histidine residues. ***For optimal iodoacetyl conjugation, we recommend Optimizer Buffer™-II.***

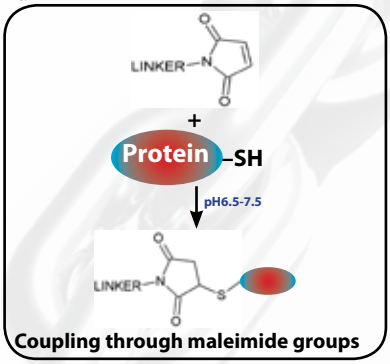
Pyridyl Disulfides

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 Double Do™ 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 provides the optimized conditions.***

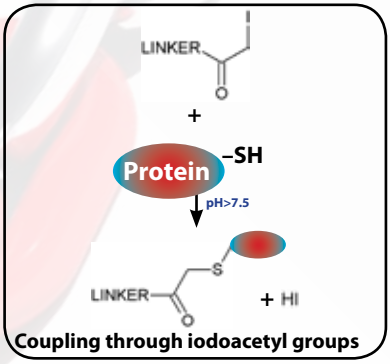
General Precautions for Sulfhydryl Reactive Reagents:

Remove reducing agents from the conjugation reaction.

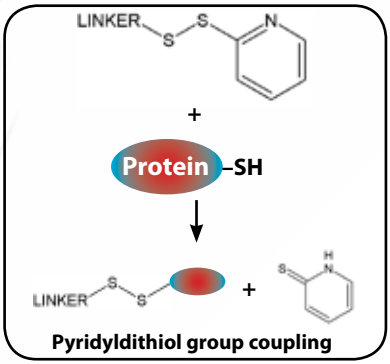
Add metal chelating agent EDTA as an anti-oxidant.



Coupling through maleimide groups



Coupling through iodoacetyl groups



Pyridyldithiol group coupling

SULFHYDRYL REACTIVE					
Cat. #	**Name**	**2nd Group**	**Cat. #**	**Name**	**2nd Group**
BC03	DPDPB	Sulfhydryl	BC19	SIAB	Amine
BC11	MBS	Amine	BC20	SMCC	Amine
BC12	sulfoMBS	Amine	BC21	SMPB	Amine
BC13	GMBS	Amine	BC22	sulfoSIAB	Amine
BC14	sulfoGMBS	Amine	BC23	sulfoSMCC	Amine
BC15	EMCH	Carbohydrate	BC24	sulfoSMPB	Amine
BC16	EMCS	Amine	BC27	Mal-PEG-NHS	Amine
BC17	sulfoEMCS	Amine	BC32	APDP	Photoreactive
BC18	PMPI	Hydroxyl			

Carbohydrate Reactive Cross-Linkers

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.

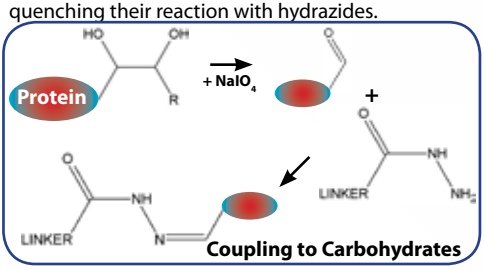
For reaction with glycoproteins, the first step is to generate carbonyl groups that react with hydrazide, under mild oxidizing conditions with sodium periodate ($NaIO_4$). At 1mM periodate and at 0°C, sialic acid residues on the glycoproteins can be specifically oxidized converting hydroxyls to aldehydes 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 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.

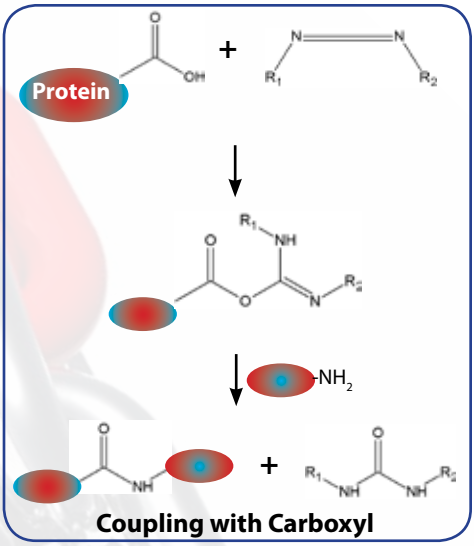
Avoid buffers containing amines, such as Tris or glycine; these buffers react with aldehydes, quenching their reaction with hydrazides.



CARBOHYDRATE REACTIVE					
Cat. #	Name	2nd Group	Cat. #	Name	2nd Group
BC15	EMCH	Sulfhydryl	BC28	ABH	Photoreactive

Carboxyl Reactive Cross-Linkers

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 pH4.5 to 7.5; however, reaction conditions of pH4.5-5.0 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 only 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. ***Optimizer Buffer™-IV 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. #	**Name**	**2nd Group**
BC25	EDC	Amine
BC28	ABH	Photoreactive

Photoreactive Cross-Linking Reagents

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 provides excellent buffer conditions.***

General Precautions for Photoreactive Reagents:

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

PHOTOREACTIVE					
Cat. #	**Name**	**2nd Group**	**Cat. #**	**Name**	**2nd Group**
BC28	ABH	Carbohydrate	BC35	sulfoHSAB	Amine
BC29	ANB-NOS	Amine	BC36	sulfoSAED	Amine
BC30	APG	Arginine	BC37	sulfoSAND	Amine
BC32	APDP	Sulfhydryl	BC38	sulfoSANPAH	Amine
BC33	BASED	-	BC39	sulfoSADP	Amine
BC34	NHS-ASA	Amine	BC40	sulfoSASD	Amine

Cross-Linking Accessories

Tube-O-DIALYZER™: Micro dialysis units

Tube-O-DIALYZER™ is a tube format dialysis device that allows for rapid dialysis with 100% sample recovery. An additional benefit is that entire cross-linking reactions can be performed in a single tube. First the protein solution can be dialyzed against an Optimizer Buffer™, or other appropriate buffer, and then, following the addition of a Double Do™ cross-linking reagent and conjugation, the uncoupled cross-linker can be dialyzed away.

The uniqu.e 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 1K, 4K, 8K, 15K and 50K. Tube-O-DIALYZER™ are available in packs of 20. Each Tube-O-DIALYZER™ is supplied with 6 appropriately sized 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.

Tube-O-REACTOR™: Complete dialysis reaction system

Tube-O-REACTOR™ is supplied with Tube-O-DIALYZER™ and micro dialysis cups and stirring balls. Each kit has sufficient components for five complete reactions.

Each Tube-O-REACTOR™ consists of 5 Medi Tube-O-DIALYZER™ or 5 Micro Tube-O-DIALYZER™ and 5 Micro Dialysis Cups, Glass Stirring Balls and 5 Floats.

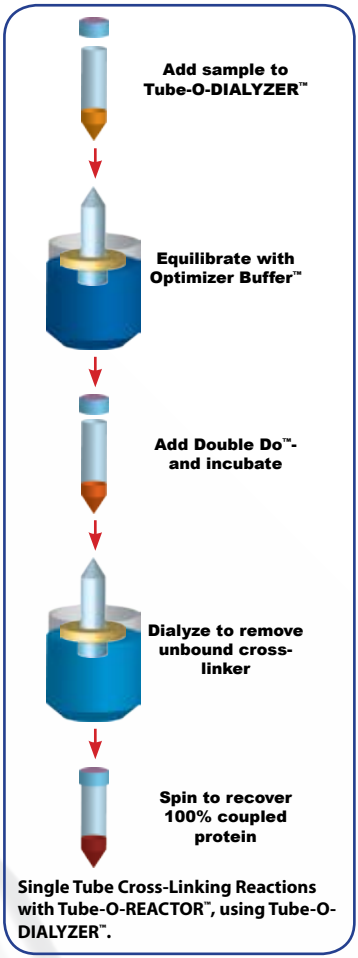
Optimizer Buffers™: For Optimal Reaction Conditions

The conjugation 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™. Use of Tube-O-DIALYZER™ is recommended for buffer exchange and best reaction results.



Single Tube Cross-Linking Reactions with Tube-O-REACTOR™, using Tube-O-DIALYZER™.

DMSO & DMF: Organic solvents for HOOK™ reagents

Vials containing anhydrous DMSO [Dimethyl sulfoxide $(CH_3)_2SO$] and DMF [N,N-Dimethylformamide $(HCON(CH_3)_2)$]. Suitable for cross-linking applications.

Sodium Meta-periodate: For oxidizing glycoproteins

Vials containing 5g sodium meta-periodate ($NaIO_4$). Suitable for oxidizing glycoproteins.

Cat. #	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
786-610	Tube-O-DIALYZER™, Micro (up to 250µl), 1000MW/ 20 units
786-611	Tube-O-DIALYZER™, Micro (up to 250µl), 4000MW/ 20 units
786-612	Tube-O-DIALYZER™, Micro (up to 250µl), 8000MW/ 20 units
786-613	Tube-O-DIALYZER™, Micro (up to 250µl), 15,000MW/ 20 units
786-614	Tube-O-DIALYZER™, Micro (up to 250µl), 50,000MW/ 20 units
786-615	Tube-O-DIALYZER™, Medi (up to 2.5 ml), 1000MW/ 20 units
786-616	Tube-O-DIALYZER™, Medi (up to 2.5 ml), 4000MW/ 20 units
786-617	Tube-O-DIALYZER™, Medi (up to 2.5 ml), 8000MW/ 20 units
786-618	Tube-O-DIALYZER™, Medi (up to 2.5 ml), 15,000MW/ 20 units
786-619	Tube-O-DIALYZER™, Medi (up to 2.5 ml), 50,000MW/ 20 units
786-620	Tube-O-DIALYZER™, Mixed, (Medi & Micro), 1000MW/ 10 units Medi, 10 units Micro
786-621	Tube-O-DIALYZER™, Mixed, (Medi & Micro), 4000MW/ 10 units Medi, 10 units Micro
786-622	Tube-O-DIALYZER™, Mixed, (Medi & Micro), 8000MW/ 10 units Medi, 10 units Micro
786-623	Tube-O-DIALYZER™, Mixed, (Medi & Micro), 15,000MW/ 10 units Medi, 10 units Micro
786-624	Tube-O-DIALYZER™, Mixed, (Medi & Micro), 50,000MW/ 10 units Medi, 10 units Micro
786-024-4K	Tube-O-REACTOR™, Micro, 4,000MW/ 5 reactions
786-024-8K	Tube-O-REACTOR™, Micro, 8,000MW/ 5 reactions
786-024-15K	Tube-O-REACTOR™, Micro, 15,000MW/ 5 reactions
786-027-4K	Tube-O-REACTOR™, Medi, 4,000MW/ 5 reactions
786-027-8K	Tube-O-REACTOR™, Medi, 8,000MW/ 5 reactions
786-027-15K	Tube-O-REACTOR™, Medi, 15,000MW/ 5 reactions
786-145A	Tube-O-Array™ Dialyzer/ 1 tray and 12 dialysis assemblies
786-145D	Tube-O-Tanks for micro size/ 1 tank
786-145E	Tube-O-Tanks for medi size/ 1 tank
BKC-16	DMF, anhydrous/ 50ml
BKC-17	DMSO, anhydrous/ 50ml
BKC-15	Sodium meta-periodate/ 5g

Double Do™ 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 relaesed by photolysis.
- **Steric Hinderance**: Bulky groups around the binding site may require reagents with longer spacer arms.

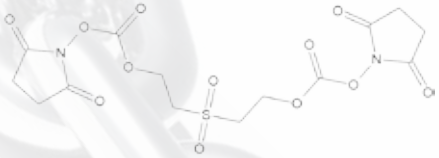
Cat. #	Double Do™ Cross-Linking Reagent	Quantity Supplied	Molecular Weight	Spacer Arm (Å)	Reactive Toward	Membrane Permeable	Water Soluble	Cleavable/ Reversible
	HOMOBIFUNCTIONAL Cross-LINKERS							
BC01	**BSOCOES** (*Bis*(2-[Succinimidooxycarbonyloxy]ethyl) sulfone	100mg	436.35	13	Primary Amines	YES	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 cross-linking***	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** Dithio*bis*(succinimidyl propionate) (Lomant's Reagent)	1g	404.42	12	Primary Amines	YES	NO	Reducing Agents (Thiols)
BC08	**DTSSP** 3,3'-Dithio*bis*(sulfosuccinimidyl propionate)	100mg	608.51	12	Primary Amines	NO	YES	Reducing Agents (Thiols)
BC09	**EGS** Ethylene glycol *bis*(succinimidyl succinate) ***Ideal for receptor ligand cross-linking***	1g	456.36	16.1	Primary Amines	YES	NO	Hydroxylamine
	HETEROBIFUNCTIONAL Cross-LINKERS							
BC11	**MBS** *m*-Maleimidobenzoyl-*N*-hydroxysuccinimide ester ***Ideal for hapten-carrier protein, toxin-antibody, enzyme-antibody cross-linking***	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*-(ρ-Maleimidophenyl) isocyanate	50mg	214.18	8.7	Sulfhydryl + Hydroxyl	nd	NO	NO
BC19	**SIAB** *N*-Succinimidyl(4-iodoacetyl)aminobenzoate ***Ideal for enzyme-antibody cross-linking***	100mg	402.14	10.6	Primary Amine + Sulfhydryl	YES	NO	NO
BC20	**SMCC** Succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate ***Ideal for enzyme-antibody cross-linking***	100mg	334.32	11.6	Primary Amine + Sulfhydryl	YES	NO	NO

Cat. #	Double Do™ Cross-Linking Reagent	Quantity Supplied	Molecular Weight	Spacer Arm (Å)	Reactive Toward	Membrane Permeable	Water Soluble	Cleavable/ Reversible
BC21	**SMPB** Succinimidyl 4-(*ρ*-maleimidophenyl) butyrate ***Ideal for enzyme-antibody cross-linking***	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 cross-linking***	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 x 5mg	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
BC27	**MAL-PEG-NHS** Maleimide PEG *N*-hydroxysuccinimide ester	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) propionamide ***Iodinatable***	100mg	446.55	21	Sulfhydryl	YES	NO	Reducing Agents (Thiols)
BC33	**BASED** *Bis*(β-[4-azidosalicylamido]-ethyl) disulfide ***Iodinatable***	100mg	474.52	21.3	Non Selective	YES	NO	Reducing Agents (Thiols)
BC34	**NHS-ASA** *N*-Hydroxysuccinimideyl-4-azidosalicyclic acid ***Iodinatable***	100mg	276.21	8.0	Primary Amines	YES	NO	NO
BC35	**Sulfo HSAB** *N*-Hydroxysulfosuccinimidyl-4-azidobenzoate	100mg	362.25	9.0	Primary Amines	NO	YES	NO
BC36	**Sulfo SAED** Sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamide)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′-propionate	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 dithio)propionate	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)

Double Do™ Cross-Linker Structures

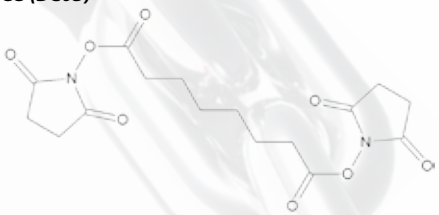
HOMOBIFUNCTIONAL CROSS-LINKERS

BSOCOES (BC01)

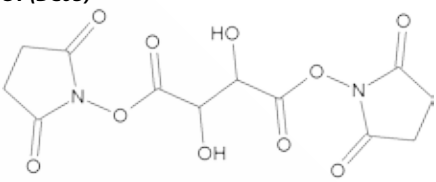


DPDPB (BC02)

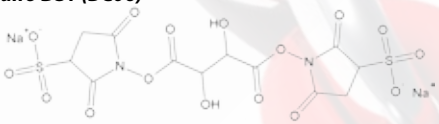
DSS (BC03)



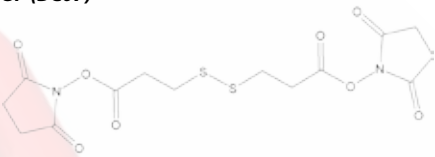
DST (BC05)



Sulfo DST (BC06)

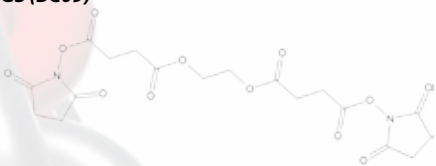


DSP (BC07)



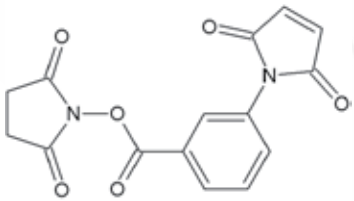
DTSSP (BC08)

EGS (BC09)

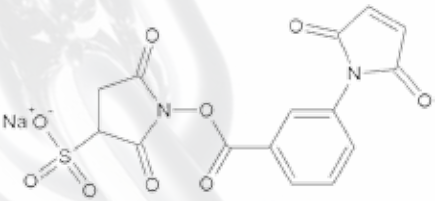


HETEROBIFUNCTIONAL CROSS-LINKERS

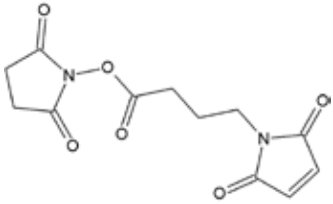
MBS (BC11)



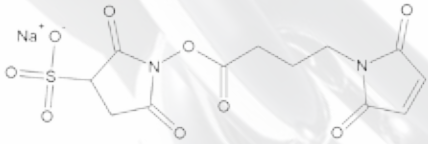
Sulfo MBS (BC12)



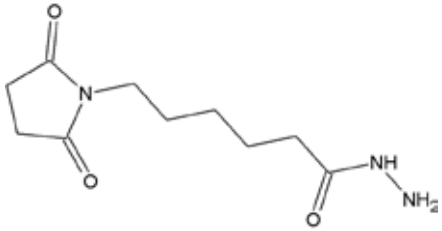
GMBS (BC13)



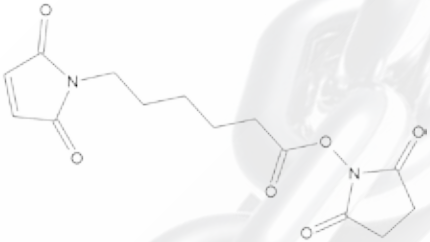
Sulfo GMBS (BC14)



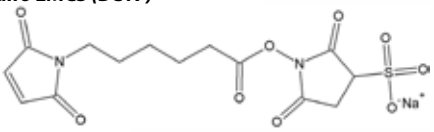
EMCH (BC15)



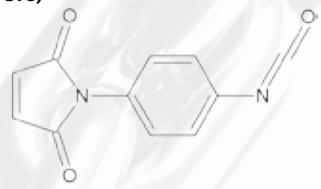
EMCS (BC16)



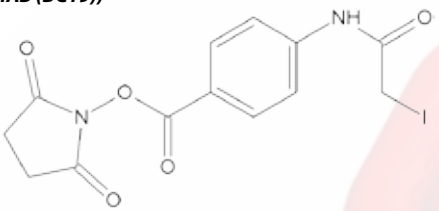
Sulfo EMCS (BC17)



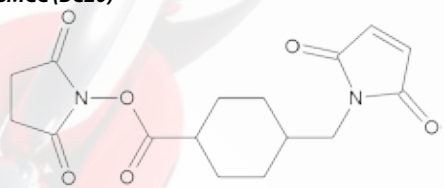
PMPI (BC18)



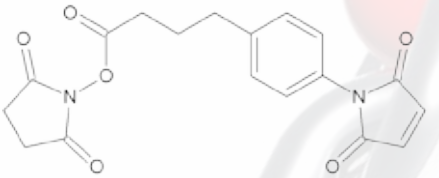
SIAB (BC19))



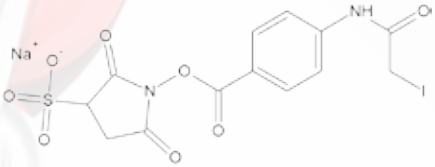
SMCC (BC20)



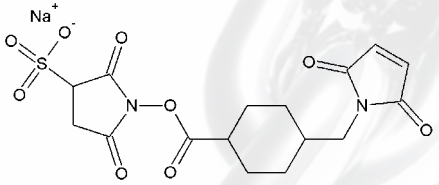
SMPB (BC21)



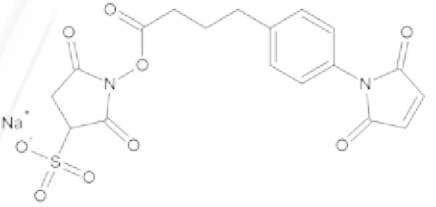
Sulfo SIAB (BC22)



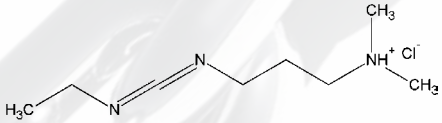
Sulfo SMCC (BC23; 786-082)



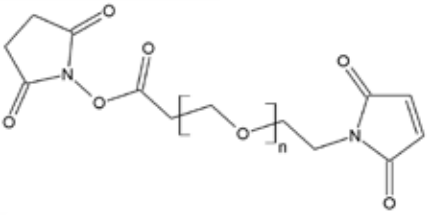
Sulfo SMPB (BC24)



EDC (BC25-1; BC25-5)

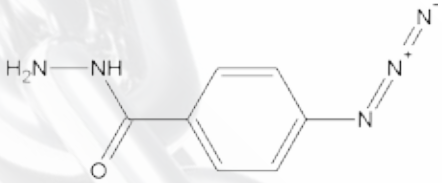


MAL-PEG-NHS (BC27)

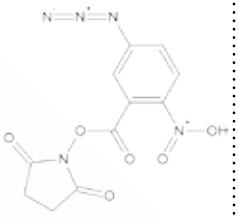


PHOTOREACTIVE CROSS-LINKERS

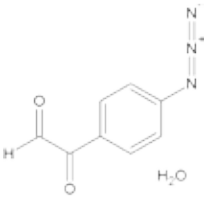
ABH (BC28)



ANB-NOS (BC29)

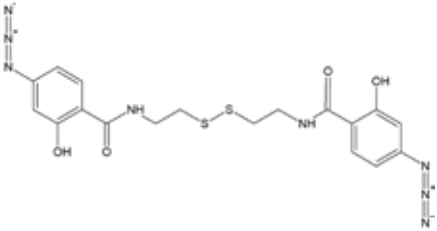


APG (BC30)

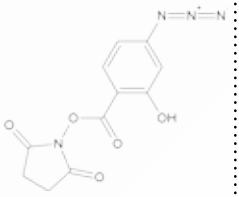


APDP (BC32)

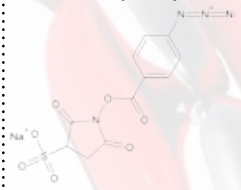
BASED (BC33)



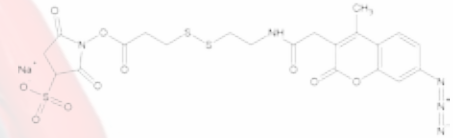
NHS-ASA (BC34)



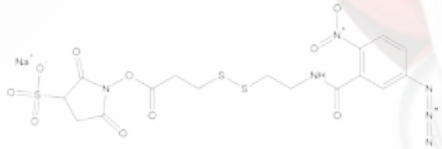
Sulfo HSAB (BC35)



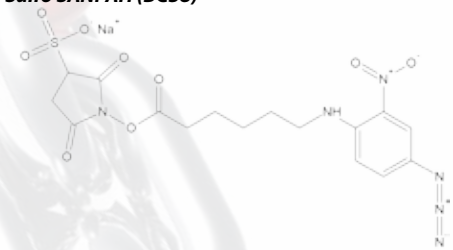
Sulfo SAED (BC36)



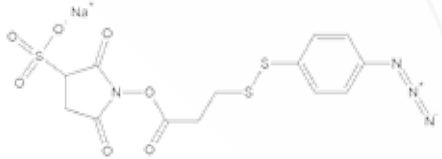
Sulfo SAND (BC37)



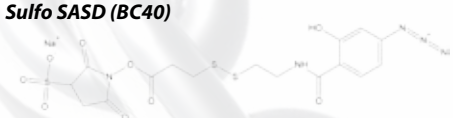
Sulfo SANPAH (BC38)



Sulfo SADP (BC39)



Sulfo SASD (BC40)

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