SoluLinK
The Conjugation Company™

NEXT GENERATION
Conjugation Reagents & Services

2007/08 Catalog & Reference Manual

Products, Kits & Services for:

- A New Generation of Conjugation Components & Products
- UV Traceable Biotinylation Reagents & Kits
- PepLinK™ Linkable Peptides
- NanoLinK™ Magnetic Separation Products
- Pre-Activated Detection Reagents
- Ask about Our Conjugation Services

100% Satisfaction Guarantee

Exceptional quality and batch-to-batch consistency are of paramount importance for all of us at SoluLinK. Consequently, SoluLinK has established strict quality control guidelines for each format of our products and each batch must pass these stringent biochemical and biological/immunological testing requirements.

However, if any product does not meet these specifications in your hands, please contact us; your concerns will be addressed quickly, and after investigation, the product will either be immediately replaced or credited for the original purchase price.

How to Order

Our Customer Service Department is available 7:00 AM – 6:00 PM (Pacific Time), Monday through Friday to take and process your orders. Orders can be placed via email, telephone, fax, mail, or online at **www.solulink.com/product_ordering.htm**.

You can either use our Product Order Form to place your order or you can send in your institution's Purchase Order form. Please be sure that the following information is included when ordering:

1. Purchase order number
2. Name of institution or company
3. Customer account number (if available)
4. Shipping address
5. Billing address
6. Catalog product number and description
7. Quantity and size required
8. Name and phone number of purchasing agent
9. Name and phone number of the end user
10. Your purchase order number (if applicable)
11. Credit card number and expiration date (if applicable)

Online Orders

Please note that for ordering on-line you will be asked to provide either a Purchase Order number or a credit card number for the order to be accepted and processed.

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www.solulink.com

Our Technical Service Scientists are available to help with assay design, to suggest relevant positive controls, and to offer troubleshooting tips.

We appreciate any feedback on our products to enable us to continue to improve our products, technical information, and our service.

SoluLinK
The Conjugation Company™

Contents

List of Technical Charts & Protocols

“I was extremely satisfied by how easily everything went together and based on these results will be hard-pressed to ever again use a maleimide. This feedback is from someone who has tried all the other commercial coupling chemistries and none work so nicely as yours.”

—Jim Coull, Chief Technical Officer
Ensemble Discovery
Cambridge, Massachusetts

About SoluLinK

SoluLinK specializes in developing and manufacturing high quality, cutting-edge conjugation reagents, assocaited products and services for *all bioscience applications*. We are committed to providing the most comprehensive panel of high quality research reagents at the most reasonable prices. Our aggressive product development program, through internal discovery, technology licensing and collaborations, produces strategic reagents for use in a variety of applications.
These include:

- detection,
- conjugation,
- flow cytometry,
- ELISA,
- immunoprecipitation,
- Western blotting,
- immunofluorescence microscopy,
- immunohistochemistry, and
- *in vitro* / *in vivo* functional assays.

With an experienced biochemistry team, SoluLinK is well positioned to offer a wide range of products and custom conjugation services to meet our customers' specific needs.

Solulink has been delivering a wide variety of quality conjugation products and custom conjugation services to prestigious life science companies and research institutes worldwide for many years. Located in San Diego, we are located in the midst of one of the nation's largest and fastest growing biotechnology areas. Our close proximity to world-renowned research institutions, such as The Scripps Research Institute, the Salk Institute, The Burnham Institute, and University of California — San Diego, catalyzes scientific exchange and collaborations.

We look forward to serving your research needs and becoming your long term research partner. We welcome your feedback and suggestions to better serve the research community at large.

SOLULINK HAS BEEN delivering a wide variety of quality conjugation products and custom conjugation services to prestigious life science companies and research institutes worldwide for many years.

Collaborate with Us

“SoluLinK seeks products with research applications to expand our product offerings. If you have reagents of commercial interest or would like information about our technology transfer process, please contact us at info@solulink.com or call our Business Development office at 888.625.0670.”

SoluLinK is committed to providing researchers with the most comprehensive and cutting-edge high quality reagents for life science research. As part of our commitment, we are interested in opportunities to develop collaborative relationships with like-minded companies and researchers. If you have developed reagents or technologies that you feel may have broad application in the life science research community, SoluLinK might be able to collaborate with you to commercialize your invention.

SoluLinK is also available to help you with SBIR and other grant applications. We welcome the opportunity to help you seek grants incorporating SoluLinK’s technology. We are also interested in working with you on grant applications that seek to develop new technologies relevant to SoluLinK’s business.

Through many technology licensing agreements with universities, research institutes, and biopharmaceutical companies worldwide, SoluLinK has developed a proven track record for successfully bringing timely and important products to market.

With this experience, SoluLinK can help ensure your reagents are commercialized quickly, effectively, and with minimal distraction to your ongoing research. Besides the financial benefit you and your institution receive from royalties from the sale of reagents, you are released from the administrative burden of providing samples and technical information to other scientists.

SoluLinK welcomes the opportunity to work with you on developing and commercializing a new product idea you have or on a grant that either incorporates SoluLinK technology or that develops a new technology.

Custom Conjugation Services

- **Too busy or focused on your core research to work on a needed conjugation?**
- **Want SoluLinK to perform a custom conjugation?**
- **Do you need to have your own antibodies, peptides, oligos, or proteins conjugated with a certain enzyme or fluorophore?**

If any of these questions fits your situation, SoluLinK can help. SoluLinK's conjugation experts can do the conjugation for you to any antibody, oligo, protein, or peptide to any other oligo, antibody, peptide or to any solid surface. Please ask, we want to help you. Just call us with a description of your conjugation project to find out about turnaround time and pricing of our custom services.

Conjugation & Custom Services Include:

- Custom conjugation of any antibody, oligo, peptide, protein to another oligo, fluor, enzyme, antibody, peptide, or immobilized on a slide or bead.
- Custom conjugation of your antibody to fluors, AP, HRP, or to solid surfaces.
- Bulk catalog reagent purchases are welcome. A review of your project by Solulink experts may find ways to improve the efficiency and yield of your conjugation project.
- If your have a unique conjugate to make several times or in larger quantities, Solulink can deliver a supply of custom conjugates to your specifications at regular intervals or in bulk quantities.
- If you have a unique chemical compound to make, Solulink's organic chemists may be able to provide the specialty synthesis to make that compound in small amounts or in bulk.

Read more about our custom conjugation services on page 77.

If you are interested in custom services for commercial applications, please contact our Technical Services Group toll free at (888) 625-0670 or email to solulink@solulink.com.

SOLULINK'S CONJUGATION EXPERTS can help you conjugate any antibody, oligo protein or peptide to any other oligo, antibody, peptide or to any solid surface.

Product Use Limitations

RUO

RESEARCH USE ONLY

Products and materials supplied by SoluLinK are for the purchaser's internal research use only. Internal research use means scientific investigation performed at the purchaser's organization.

You may not use our materials for any diagnostic, therapeutic, or commercial purpose without a license from SoluLinK. Commercial purpose means any use for which you are compensated, including:

- **any use in the manufacture of a product, information or data for sale or in rendering a service to a third party**
- **any resale, repackaging or other transfer for consideration**

Commercial licenses are available, please inquire.

Trademarks & Patents

Patents

US 6,800,728 Hydrazine-based and carbonyl-based cross-linking reagents, October, 2004.

US 6,686,461 Triphosphate oligonucleotide modification reagents and uses thereof, February, 2004.

US 6,911,535 Biomolecule/Polymer Conjugates, June, 2005.

US 7,102,024 Ternary Biomolecule/Polymer/Surface-Based Immobilization Systems.

Novel Hydrazone- and Oxime-Based Fluorescent and Profluorescent Reagent, Application submitted 2004.

In-licensed exclusive worldwide support patents

US 5,679,778 Molecule labeling using 2-hydrazinopyridine derivatives.

US 5,206,370; US 5,420,285;US 5,753,285; Certain pyridyl hydrazines and hydrazides useful for protein labeling.

Trademarks

The following are trademarks of SoluLinK, Inc:

- SoluLinK™
- ChromaLinK™
- NanoLinK™
- PepLinK™
- HydraLinK™
- The Conjugation Company™

Other trademarks and patents of other companies are referenced in this catalog.

These products are for research use only, may be subject to license, and are covered by US and EU patents. Refer to page 6 for more information.

Frequently Asked Questions

Q: What types of custom conjugations does SoluLinK perform?

A: SoluLinK's versatile linking chemistry allows for a wide range of linking schemes. We can link your protein, oligo, peptide or other bio-molecule to another protein, DNA, RNA, reporter enzyme, fluorescent dye, polysaccharide, or solid surface such as a bead, slide or plate. Other linking schemes are also possible, please contact us to inquire about additional methods.

Q: I've tried other kits; is it easy to use SoluLinK conjugation kits?

A: SoluLinK's kits and catalog products have been used by hundreds of investigators like yourself for years. One key advantage SoluLinK conjugation products have is that our linker has a built in detection component making it easy to verify that the conjugation has taken place.

Q: Can SoluLinK order the materials required for my conjugation?

A: SoluLinK can directly order all of the materials required for your conjugation project including oligos, peptides, antibodies, etc. *Simply specify the vendor and product number of the product that you would like to use and SoluLinK will do the ordering for you.*

Q: How much protein does SoluLinK require to perform the conjugation?

A: We can conjugate as little as 100 µg of your protein or bio-molecule! Larger quantities of starting material will offer better yields, however. Typically maximum yields are obtained with >500 µg of starting material, depending on the conjugate purification method employed.

Q: How long will it take to receive my conjugate?

A: Turn-around time is approximately two weeks from the date all starting materials are received.

Q: I would like additional analysis performed on my conjugate, is this possible?

A: We can perform additional analyses of your conjugate which are not routinely performed for the Certificate of Analysis. These techniques include SDS-PAGE, enzymatic activity analysis, precise level of DNA or RNA incorporation, and others. Please see the Custom Pricing section for additional information and pricing.

Q: My antibody is currently in ascites fluid/serum/tissue culture supernatant. Can SoluLinK purify my antibody prior to conjugation?

A: Absolutely! For an additional charge, SoluLinK will isolate your antibody from a crude preparation and conjugate it to the molecule of your choosing. Please note that >500µg of antibody will need to be supplied to obtain sufficient material for downstream conjugation. Please see the Custom Pricing section for more information.

Q: How will the final conjugate be supplied?

A: As requested, conjugates can be delivered in any desired buffer and concentration.

Q: I am using my conjugate for *in vivo* applications. Can I request that no preservatives be added?

A: Yes. Please specify that you would like no preservatives added when you place your order.

Finally.

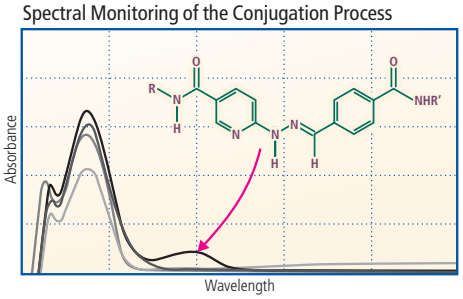
Simple and Stable Conjugation Chemistry!

The SoluLink Conjugation System

Finally, a small molecule conjugation chemistry that is easy to use, stable in solution, makes better conjugates, and is applicable to almost any conjugation problem! SoluLink chemistry is highly selective, stable in solution, and not susceptible to non-specific binding, making it superior to conventional methods.

- **Stable modification**
- **Efficient conjugations**
- **Easier to use**
- **Quantifiable conjugates**

Spectral Monitoring of the Conjugation Process



- **3x Better performance**
- **10x Better stability**
- **10x Reproducibility**
- **5x Higher yields**
- **5x Ease of use**
- **3x Lower unit cost**

Solulink's proprietary new chemistry connects biomolecules to each other or to solid surfaces and uniquely allows each to retain its original capabilities, yet synergistically take on new functionality as a result of the combination. Our conjugation chemistry has been engineered to conjugate, label and immobilize biomolecules such as proteins, antibodies, peptides and oligonucleotides — including DNA and RNA amplicons. The benefits of Solulink's innovative technology, increased utility, and ease-of-use are evident in our new product: ChromaLink Biotin 354S. This novel, widely-used reagent allows scientists to simultaneously incorporate and spectrophotometrically measure biotin — with one reagent.

Already being used by prestigious research and commercial organizations worldwide in next-generation biomedical assays, detection systems, and protein arrays, our technology is available for commercial use through supply agreements or through licensing. Learn more about Solulink and our products by visiting our website, www.solulink.com or email us at info@solulink.com.

For more information about our reagents and services, visit our website or call us at 858.625.0670.

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SoluLinK Biosciences

The Conjugation Company™

Conjugation Products

"We have had trouble doing conjugations with the previous chemistries then after conjugation they had a tendency to fall part over time. We were extremely satisfied by how easily SoluLinK linkers worked and the resulting stability was far superior to previous linkers."

SoluLinK
The Conjugation Company™

Conjugation Background

Introduction

The Standard bioconjugation cross-linkers have been used for decades. So the question arises, what is wrong with those cross-linking chemistries and how is SoluLinK different? SoluLinK firmly believes previous cross-linkers (SMCC, SPDP, BS^3 and versions thereof) suffer from obvious limitations that are often overlooked. SoluLinK provides its customers a more efficient, stable and quantifiable bioconjugation chemistry.

The limitations of common first generation conjugation technologies include:

- No traceable signature that allows monitoring of conjugate formation.
- Old technologies are not inert and continue to react (post-modification) with biologically derived $–NH_2$ and –SH groups and often lead to inefficient and unwanted homoconjugate formation.
- Common heterobifunctional linkers have short-term stability in aqueous environments and often possess two hydrolysis-susceptible functionalities (–NHS and –Maleimide).
- Many of these conventional cross-linkers often lack traceability of the modification process which leads to biological over-modification and a measurable loss of binding affinity, avidity, and/or function.
- Conjugate yields and batch-to-batch consistency are difficult to optimize and reproduce without traceability of the entire process.
- Typical linkers often require an additional activation step with harsh reducing agents that can impact biological performance.
- They are incompatible with automated DNA and peptide synthesis instruments.

For these and other reasons SoluLinK felt it needed to develop a more forward-looking and innovative chemistry to address these and other limitations with the use of common cross-linkers. The following pages reveal many techniques and performance benefits to using SoluLinK's bioconjugation chemistry. We hope our products can help you, the customer, with simpler and more efficient methods by eliminating the frustration of making and evaluating your own bioconjugates.

Conjugation Background

What Makes SoluLinK Conjugation Chemistry Different?

Traceability	The bis-aryl hydrazone bond formed with HyNic absorbs at 354 nm with a molar extinction coefficient of 29,000.
Reproducibility	Biomolecule modification can be quantified for greater batch-to-batch reproducibility.
Linker Stability	Biomolecules modified with hydrazines and aldehydes have extended stabilities.
Bond Stability	Bis-aryl hydrazones are stable to temperatures up to 94°C and a wide pH range, pH 2-11.
Specific	SoluLinK Bioconjugation chemistry assures formation of heteroconjugates, with no formation of homoconjugates. Reactive moieties on modified biomolecules are inert and do not react with protein functional groups.
Aqueous Chemistry	Reactions are carried out in aqueous buffered solutions with high efficiency.
Versatility	Our chemistry has been engineered to conjugate and immobilize proteins, oligonucleotides, peptides and carbohydrates.
Solid Phase Synthesis	Linkers can be incorporated in both peptides and oligonucleotides during solid phase synthesis.

Conjugation Background

“We recently were able to link our TLR agonist to a phospholipid via your Solulink method. These hybrid molecules can be incorporated into nanoparticle formulations with the great advantage that the incorporation of the conjugate into the nanoparticle can be quantified via UV absorbance mearurements which do not interfere with the other components of the complex particle. I highly recommend your linker method for this application.”

—Wolfgang Wrasidlo, PhD
Director of Medicinal Chemistry
Moores Cancer Center
University of California, San Diego

Bioconjugates: Key Players in the Life Sciences

Bioconjugation is the joining of two biomolecules, each with its own separate function, and uniting them into a single species possessing both functions — thus creating a 1 + 1 = 3 synergy. Monoclonal antibodies have long been used in both diagnostics and therapeutics. In the field of therapeutics, monoclonal antibodies have had a long history (since the 1980s) of success for the treatment of many different types of clinical disease[1]. More recently, monoclonal antibodies have been conjugated to other biomolecules and used as therapeutically enhanced agents. For example, therapeutic anti-cancer drugs have been conjugated to anti-tumor antibodies to efficaciously target and deliver drugs to the tumor site[2, 3].

In diagnostics, ELISAs (Enzyme-Linked Immunosorbent Assays) are among the most widely used conjugate-based method for detecting disease-related antigens in both research and clinical environments. Bioconjugates are also used in many common immunohistochemical techniques that employ fluorescent or enzyme antibody conjugates to spatially localize and identify cellular components. *In situ* methods have been widely used to detect various cellular components that include:

- cytoplasmic and nuclear proteins,
- genomic DNA,
- RNA (including mRNA, rRNA, and miRNAs), and
- structural components such as organelles and microfilaments.

Fluorescence *in situ* hybridization (FISH) and immunohistochemical (IHC) methods are now considered an indispensable tool for the clinical pathologist. Antibody conjugates are used to bind specific cells that target cell surface markers. These conjugates are used to both enumerate and enrich specific cells from heterogeneous populations with the aid of FACS (Fluorescence Activated Cell Sorters).

In more recent developments, bioconjugates have established themselves as important tools in the emerging fields of genomics and proteomics by playing a critical role in the generation of high throughput, quantitative gene expression and protein microarray profiling data. Recently, significant interest[1-4] has emerged in the use of cationic peptides for the delivery of proteins, nucleic acids and drugs into the cytoplasm of cells. The exact mechanism of action for these cell-penetrating peptides (CPPs) has not been fully elucidated but their utility as bioconjugates has led to extensive use for cytoplasmic drug and antibody delivery. More specifically, various peptide sequences that include TAT (and Tat-like sequences) are being conjugated to numerous other biomolecules including siRNA, antibodies, and even nanoparticles for subsequent transport across the cell membrane. In other developments, peptide-carrier conjugates are being widely used as immunogens for the generation of tens of thousands of individual antibodies targeting the approx. 32,000 known proteins encoded by the human genome. Bioconjugates continue to forge new frontiers and it is clear that their growing applications will continue to impact the fields of biology, medicine, and the life sciences in general.

Conjugation Background

What Is Bioconjugate Chemistry?

In simple terms, biomolecules exist and function in aqueous environments, therefore bioconjugate chemistry is primarily about "chemistry in water." Any suitable bioconjugate chemistry must be compatible with such an environment, while at the same time preserving the biological activity or function of any biomolecule. Conjugates are generally formed through the introduction of separate functional groups into each of the two biomolecules and subsequently mixed together to form the desired bioconjugate.

The SoluLinK Bioconjugation Process

The basic SoluLinK conjugation process, illustrated in Figure 1, shows the conjugation of two amine-containing biomolecules, in this case, two different proteins. Biomolecule 1 is activated with functional linker A [S-HyNic], and Biomolecule 2 is activated with functional linker B [S-4FB]. Once modified, the proteins are desalted and quantified using Bradford, BCA, or other suitable methods such as absorbance at 280 nm, and immediately followed by quantitatively measuring the degree of linker modification. Once modification is complete, simple mixing of the two biomolecules allows the two linkers to selectively react with each other forming the conjugate. Biomolecule concentration and the specific reaction between the linkers then becomes the main driving force behind conjugate formation, generally at pH 4.5 to pH 8.0.

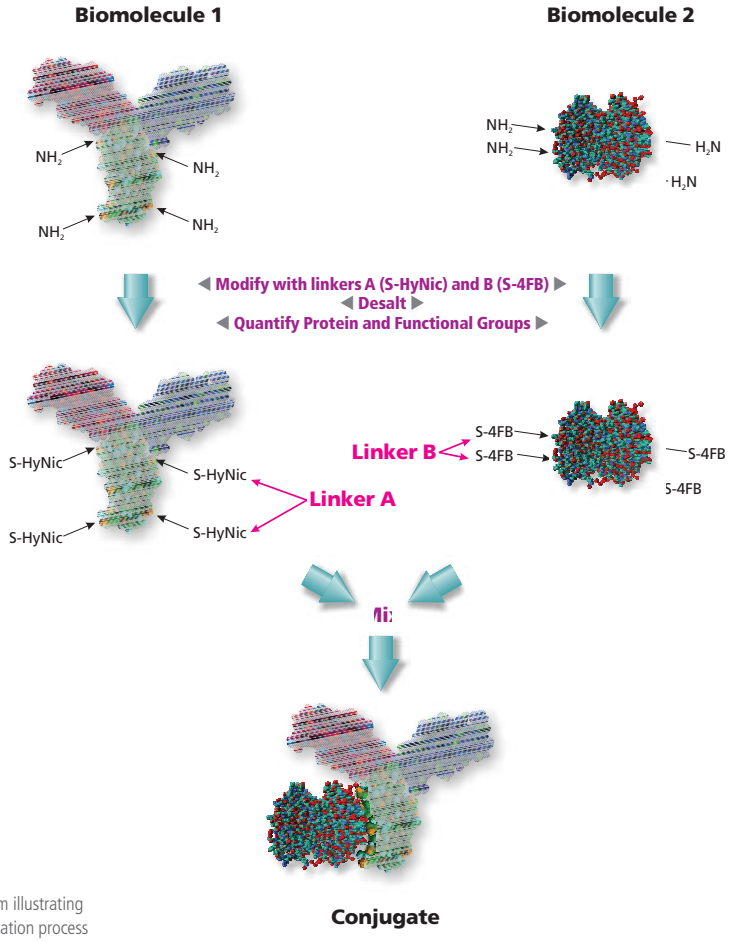
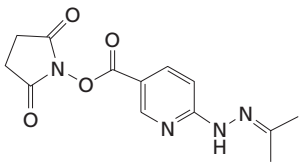


FIGURE 1 • Diagram illustrating the SoluLinK conjugation process

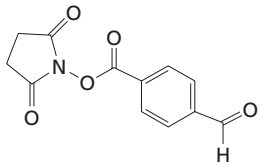
"In our hands, Solulink linkers have proven flexible and soundly designed products well tailored to customized production of bioconjugates. The conjugation chemistry has wide application, is simple to perform, stable in solution and can be conveniently prepared in stock for later usage. Solulink products are a valuable component of our laboratory toolbox."

—John Mountzouris, Ph.D.
Abgent
San Diego, California

Conjugation Background



S-HyNic (4)
Succinimidyl 6-hydrazinonicotinamide acetone hydrazone



SF-4B (5)
Succinimidyl 4-formylbenzoate

FIGURE 3 • SoluLinK's amine-reactive modification reagents, S-HyNic (SANH) (aromatic hydrazine) for incorporation of 2-hydrazinonicotinamide (HyNic) moieties and S-4FB (SFB) for the incorporation of 4-formylbenzamide moieties (4-FB).

What is the Ideal Bioconjugate Linking Chemistry?

Bioconjugates are made using functionalized linkers. At this point, it might be instructive to list some of the criteria that might define ideal functional linkers:

- Linkers should be introduced into a biomolecule by an efficient reaction
- Attachment of linkers should be quantifiable
- Reaction between the linkers should lead to a traceable conjugate 'signature'
- Reaction between linkers should form stable covalent bonds
- Reaction between the linkers should be rapid and efficient
- Post-modification, linkers should remain reactive to each other (aqueous media) but inert to other biological moieties (–NH2, –SH, or –COOH groups) long-term

In specific terms, our bioconjugation chemistry is based on the formation of a stable, UV-traceable bis-aryl hydrazone bond between two biomolecules. This bond is created when a Schiff base is formed by reaction of a hydrazinonicotinamide-modified (HyNic) biomolecule (1) with a formylbenzamide-modified (FB) biomolecule (2) to form a stable bis-aryl hydrazone bond as outlined in Figure 2. This unique covalent bond is the first known example of a stable Schiff base that does not require reduction to stabilize the bond.

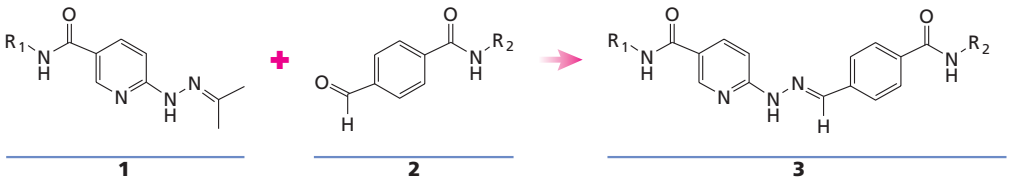


FIGURE 2 • Scheme outlining the specific reaction between SoluLinK's aromatic hydrazine linker, 6-hydrazinonicotinamide **(1)**, and the aromatic aldehyde linker, 4-formylbenzamide **(2)** used to create a stable bis-aryl hydrazone bond **(3)**. R1 and R2 correspond to the two biomolecules.

SoluLinK's S-HyNic (SANH) and S-4FB (SFB) Linkers

At the core of SoluLinK's technology are the two functional linkers S-HyNic (4) and S-4FB (5) as seen in Figure 3. S-HyNic was developed to incorporate 6-hydrazino-nicotinamide moieties into amine-containing biomolecules via an activated N-hydroxysuccinimide ester. This heterobifunctional linker possesses unique chemical properties. For example, S-HyNic is the first linker capable of incorporating a nucleophile on a biomolecule without the need for an additional activation step. This is possible because the nucleophilic hydrazine is protected as an alkyl (acetone) hydrazone that provides it with long-term stability either as a solid or liquid (organic solvents). This protection also maintains the stability of the hydrazine moiety for long periods of time in aqueous media through a dynamic equilibrium between the acetone-protecting group and the hydrazine.

Typical protein cross-linkers such as maleimide-thiol linkers are highly susceptible to aqueous hydrolysis and therefore must be used immediately after modifying the protein. This is not the case with S-HyNic-modified proteins. The functional HyNic group remains stable to aqueous hydrolysis yet reactive toward aromatic aldehydes for months at 4°C or colder as illustrated in Figure 4.

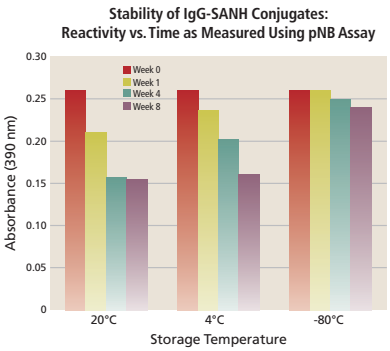


FIGURE 4 • Stability of hydrazinopyridinyl moieties conjugated to IgG as measured by reaction with a UV-traceable reagent (p-nitrobenzaldehyde) otherwise known as p-NB reagent. As seen in the graph, S-HyNic (SANH)-modified IgG stored at 4°C or at -80°C for 4 weeks preserves its hydrazine reactivity toward an aromatic aldehyde by 80% and 95%, respectively.

Conjugation Background

S-4FB is SoluLinK's other functional linker which complements and reacts with S-HyNic-modified biomolecules to form conjugates. This amine-reactive functional linker incorporates aromatic aldehydes on proteins and other biomolecules. Once incorporated, it can react with HyNic-modified biomolecules to form stable conjugates. The hydrazone bond formed between these two functional linkers is acid catalyzed. The pH range for optimal conjugation kinetics is between 4.5 and 5.0, however the conjugation will proceed even up to pH 8.0, albeit more slowly. The aromatic aldehyde is known to be even more stable to long-term storage (in aqueous solutions) than S-HyNic-modified proteins. In our hands, this linker has remained reactive to HyNic groups for up to a year when stored at -80°C in aqueous solution with virtually no loss of performance.

The aqueous stability of these linkers provides SoluLinK's bioconjugation chemistry with greater flexibility and convenience than other available conjugation chemistries. Proteins and other biomolecules can be modified with either reagent (S-HyNic or S-4FB) and subsequently stored at 4°C (or less) until one needs to make the conjugate. This long-term stability is one of the unique and distinguishing features of SoluLinK chemistry over other common cross-linking technologies. The conjugation reaction can be performed weeks after the modification step. Scale-up becomes easier knowing modified proteins are stable in storage while the MSR is determined or smaller scale pilot conjugation studies are conducted.

Advantages of SoluLinK Chemistry

Traceability is perhaps the greatest advantage of using SoluLinK's bioconjugation chemistry, and is directly engineered into our chemistry. SoluLinK conjugation chemistry is the only linking chemistry that permits both the protein modification step and the conjugation step to be quantitatively monitored or traced via hydrazone bond formation.

Molar Substitution Ratio (MSR)

This quantitative feature allows a user to determine the number of linkers attached to any given protein or biomolecule and is commonly referred to as the Molar Substitution Ratio or MSR. The MSR is readily determined using a simple quantitative reaction and a UV-scan. The unique traceability of our hydrazone chemistry is illustrated in Figure 5. As can be seen in the figure, SoluLinK uses p-nitrobenzaldehyde (p-NB) (6) to readily form a UV-traceable hydrazone bond ($Å_{390}$) on reaction with HyNic groups incorporated after modification of the protein. Conversely, 2-hydrazinopyridine (2-HP) (7) is used to form UV-traceable hydrazone

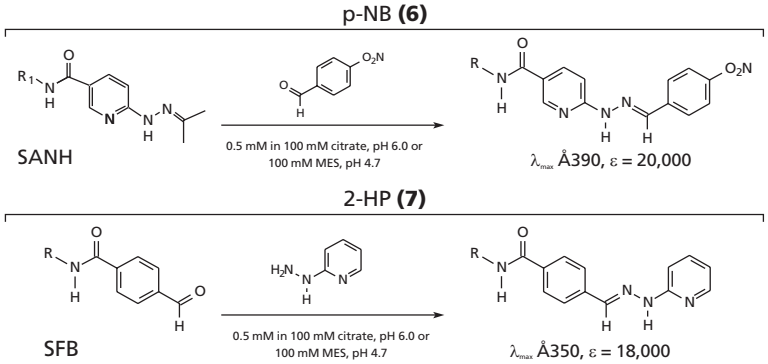


Figure 5 • Reaction schemes for the quantification of 2-hydrazinopyridyl and benzaldehyde

Conjugation Background

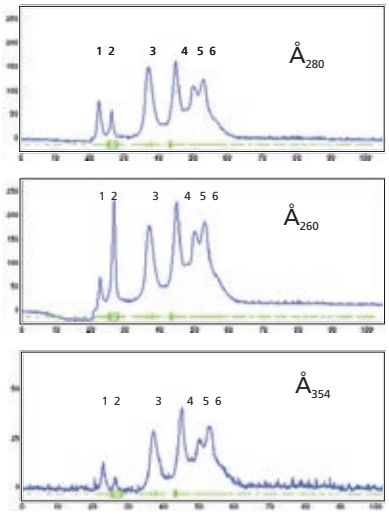
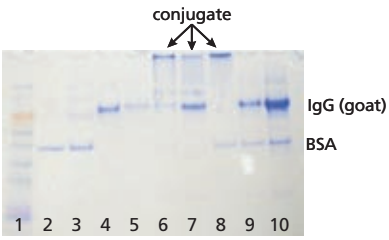


FIGURE 6 • Chromatographic elution profiles of a protein-oligonucleotide conjugate (Q-Sepharose @ 280 nm, 260 nm, and 354 nm) made using S-HyNic (SANH) and S-4FB (SFB) reagents. The unique 354 nm signature (bottom trace) along with the 260 nm elution profile (center trace) unambigously confirmed peak 3 as the desired 1:1 oligo-protein heterodimer with peaks 4, 5, and 6 identifying more highly modified oligonucleotide heteropolymers. Final conjugate results were confirmed by SDS-PAGE analysis (data not shown).



1. Molecular weight protein marker
2. BSA
3. BSA-HyNic
4. IgG
5. IgG-4FB
6. IgG-HyNic + IgG-4FB (1:1)
7. BSA-HyNic + IgG-4FB (1:3)
8. BSA-HyNic + IgG-4FB (3:1)
9. BSA + IgG-4FB (1:1)
10. BSA-HyNic + IgG (1:1)

FIGURE 7. CONJUGATION of HyNic-modified BSA (lane 3) with 4FB-modified IgG (lane 5). Reactions of 1:1, 1:3, and 3:1 molar equivalents of BSA-HyNic and IgG-4FB are seen in lanes 6, 7, and 8, respectively. Lanes 9 and 10 are control reactions wherein unmodified BSA is reacted with IgG-4FB and BSA-HyNic is reacted with unmodified IgG, respectively.

bonds ($Å_{350}$) on reaction with benzaldehyde groups. Although the assay is destructive, it only requires as little as 20 µg of protein to determine the MSR of any modification reaction. The hydrazone bonds formed by these reactions have a relatively high molar extinction coefficient that allows the degree of modification to be quantified using a simple absorbance measurement.

Precise measurement of the MSR provides the user with unparalleled control and monitoring of the modification reaction. Fine-tuning the MSR is critical to generating high conjugate yields cost-effectively without negatively impacting the activity or function of the protein or biomolecule. In our experience, traceability and judicious control of the MSR has also allowed SoluLinK chemistry to generate higher recovery yields of more defined conjugates (1:1 heterodimers). Cost factors are often in play when trying to maximize conjugate recovery yields during purification and precise measurement of the MSR is key to obtaining higher yields. Finally, quantitative traceability of the conjugation process insures batch-to-batch reproducibility and consistency.

In our experience, traceability of conjugate formation can be just as important and useful as traceability of the modification reaction (MSR). The reaction between an aldehyde and a hydrazine-modified biomolecule also leads to *traceability of conjugate formation*. A conjugate-generated UV-signature is often and routinely employed to identify which fractions to collect or pool during the conjugate purification process. This unique hydrazone-mediated signature is only present when a conjugate has been formed. This traceable signal helps the user monitor conjugate fractions during the chromatographic purification process.

For example, as seen in Figure 6, the absorbance of the hydrazone (354 nm) can be used to identify and characterize various protein-oligonucleotide conjugate species. As seen in the figure, use of the hydrazone signature at 354 nm from a typical elution profile (Q-Sepharose) in conjunction with other monitored wavelengths [280 nm (protein) and 260 nm (oligo)] is extremely useful in confirming which fractions to collect and analyze. These profiles demonstrate the utility of having our unique 354 nm signature in confirming conjugate bearing peaks.

Examples

Protein-Protein Conjugates

Often the most difficult biomolecules to conjugate are proteins due to the stringent conditions required to maintain their biological function. SoluLink routinely uses S-HyNic (succinimidyl 6-hydrazinonicotinate acetone hydrazone) and S-4FB (succinimidyl 4-formylbenzoate) to modify the ε-amino group of lysines in proteins so as to introduce HyNic and 4-FB moieties, respectively. SDS-PAGE analysis of crude, unpurified reactions readily illustrates single step conjugation of HyNic-modified BSA with 4FB-modified IgG (Figure 7). Stable conjugate formation occurs under mild conditions and without reducing agents.

Conjugation Background

Protein-Oligonucleotide Conjugates

The hydrazine-aldehyde bioconjugate couple is ideal for preparing oligo-protein conjugates as illustrated in Figure 8. In this experiment, IgG is reacted with 7.5 and 15 equivalents of S-HyNic (SANH) to incorporate hydrazine moieties on IgG. Following desalting of the reaction, the hydrazine-modified IgG (IgG-NHNH2) was reacted with 10 equivalents of 5′-aldehyde modified oligonucleotide in 0.1 M MES, 0.9% NaCl, pH 4.7 for 2H. SDS-PAGE gel-shift analysis of the conjugate formed indicates that close to 100% of the modified-IgG was linked to oligonucleotide as determined by a nearly quantitative ‘upward’ gel-shift of the original starting IgG (lane 3, CB-staining). This slight upward gel shift is always seen when conjugating oligonucleotides with IgG. UV-backshadowing of the gel also confirms the incorporation of UV-absorbing oligonucleotide conjugated to the antibody (lane 3, UV-backshadowing). Subsequently, the proteins were transferred to a PVDF membrane and hybridized with a 5′-fluorescein-labelled oligonucleotide complementary to the first oligo-nucleotide. Hybridization experiments demonstrated that the fluorescein-oligonucleotide was hybridized to the IgG conjugate (lane 2 and 3, Southwestern). SoluLinK has routinely conjugated 75-mer 5′-aldehyde modified oligonucleotides to hydrazine-modified antibodies with excellent yields (60–75%) as based on protein recovery.

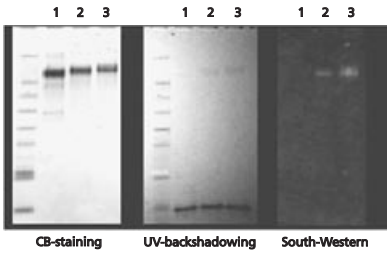


Figure 8 • IgG was first modified to incorporate hydrazine moieties by reaction with S-HyNic (SANH) 7.5 equiv and 15 equiv. (lanes 2 and 3 respectively) followed by incubation of the modified IgG with 10 equivalents of a 5′- aldehyde-modified oligonucleotide (22-mer). The gel on the left was stained with Coomassie blue (pre-stained markers) to detect protein and the same gel was visualized by UV-backshadowing (center) demonstrating the presence of excess oligonucleotide (bottom of gel) and conjugated oligonucleotide associated with the IgG (top of gel, lane 2, 3). The gel on the right is a southwestern blot wherein the protein was transferred to a PVDF membrane and a complementary 5′-fluorescein-labelled oligonucleotide was hybridized to the oligo-IgG conjugate. The Southwestern clearly demonstrates that the biological activity (hyridizability) of the oligonucleotide is retained following conjugation to the IgG.

Oligonucleotide-Peptide and Oligonucleotide-Hapten Conjugates

SoluLinK chemistry can also be used to prepare oligo-peptide and oligo-hapten conjugates. In order to make an oligo-peptide conjugate we first synthesized a 25-mer bearing a 5′–aldehyde using standard phosphoramidite chemistry. The structure of this phosphoramidite can be seen in Figure 9 (Panel A). Using this reagent a 5′-aldehyde 25–mer oligonucleotide was prepared on an automated DNA synthesizer and purified by HPLC (Figure 9, Panel B). The 5′-aldehyde oligonucleotide was tested by reaction with hydrazino-stilbazole. Success-ful conjugation of the oligo to the hapten led to a readily detectable oligo-hapten conju-gate (Figure 9, Panel C, lane 2).

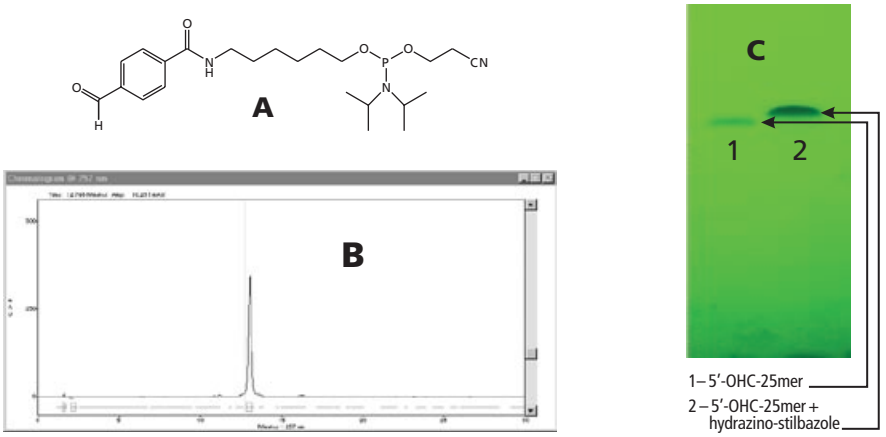
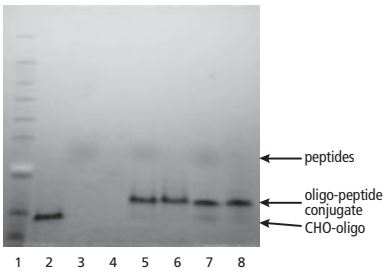
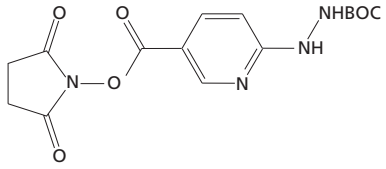


Figure 9 • To further increase the utility and ease of using SoluLinK technology, we developed a 4-formylbenzamide phosphoramidite (A) that can be incorporated on the 5′-end of deoxy- and ribo-oligonucleotides. This phosphoramidite is incorporated during solid phase synthesis using standard conditions that result in yields equal to incorporation of amino moieties. (A) Structure of SoluLinK’s aromatic aldehyde, 4-formylbenzamide-C6-phosphoramidite, (B) analytical HPLC of purified 5′-4-formylbenzamide oligonucleotide and (C) PAGE analysis of 5′-4-formylbenzamide oligonucleotide (lane 1) and the reaction of the 5′-4FB oligo with a small molecule hydrazine, 4′-trans-hydrazinostilbazole (Fluka Chemicals) demonstrating complete quantitative conversion of aldehyde to hydrazone conjugate.

Conjugation Background





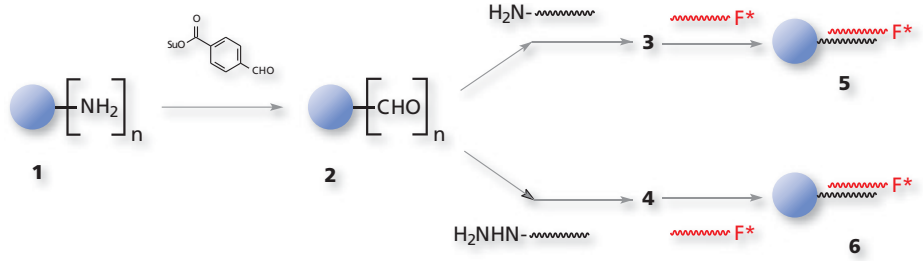
6-BOC-HNA-OSu
(Succinimidyl 6-BOC-hydrazinonicotinate)

1 – Molecular weight protein marker
2 – CHO-ODN
3 – Hydrazine Peptide 121
4 – Hydrazine Peptide 122
5 – Hydrazine Peptide 121 + CHO-ODN
6 – Hydrazine Peptide 122 + CHO-ODN
7 – 4 (incubated at 94°C for 2 hr)
8 – 5 (incubated at 94°C for 2 hr)

FIGURE 10 • A formylbenzamide-modified oligonucleotide (5′-aldehyde) was synthesized by solid phase automated DNA synthesis and reacted with two C6-HNA modified peptides (lane 3, 4). Reaction of the two modified biomolecules led to an oligo-peptide conjugate (lanes 5,6). The thermal stability of the hydrazone bond was rigorously tested by heating the peptide-oligo conjugate at 94°C for 2 hours (lanes 7,8).

A 15-mer peptide (Figure 10) was synthesized using succinimidyl hydrazone reagent (6-Boc-HNA-OSu) during solid phase peptide synthesis to directly incorporate the hydrazine moiety. Simple addition of the hydrazine-modified peptide to the aldehyde-modified oligonucleotide (Figure 10, lane 2) directly yielded the peptide-oligonucleotide conjugate (lanes 5 and 6) without the requirement of reducing agents. The thermal stability of the hydrazone bond was rigorously tested by heating the peptide-oligo conjugate at 94°C for 2 hours (lanes 7, 8) and confirms the stability of the hydrazone bond linking two biomolecules together.

Conjugation of Biomolecules to Solid Phases: Oligonucleotides and Antibodies



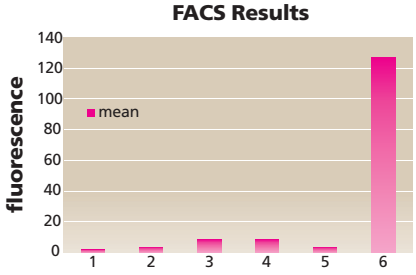


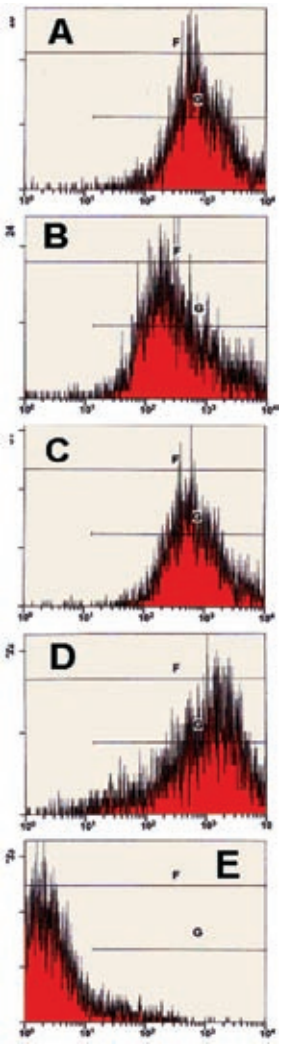
FIGURE 11 • Amino-modified latex beads (1) were modified to incorporate FB moieties (2) using S-4FB (SFB). The beads were then conjugated to HyNic-modified oligonucleotides (4). Following several washes, the oligo-modified beads were hybridized to a 5′-fluorescein modified complement and analyzed by flow cytometry. In the control experiment, amino-modified oligonucleotide (3) was incubated with FB-modified beads. As seen by the FACS data, HyNic-modified oligonucleotides conjugated to the FB-beads hybridize to their complement (6) to yield a signal to noise ratio (S/N) as high as 50:1.

The stability of SoluLinK's bioconjugation chemistry is particularly suited to the immobilization of biomolecules on solid phases, including latex beads. The reasons are related to convenience, reproducibility, and signal to noise (S/N). For example, the inherent stability of aldehyde-modified latex beads allows them to be activated months ahead of their actual use and then stored in aqueous media. Stored, ready-to-use beads offer convenience and reliability because larger batches can be prepared at any time without having to prepare a fresh lot each time one wishes to immobilize a given biomolecule. Modifi-

Conjugation Background

cation of beads is simple and efficient and starts with the modification of the amino-modified latex surface with S-4FB to incorporate formylbenzamide groups (FB-beads). The beads are then washed to remove excess reagent. Once modified, the FB-beads are ready for use. Simple mixing of any hydrazine-modified biomolecule with the FB-latex beads leads to efficient covalent immobilization. In Figure 11, we demonstrate the successful immobilization of an amino-modified oligonucleotide and subsequent hybridization of its complement.

SoluLinK has also demonstrated efficient conjugation of an *antibody to aldehyde-modified beads* with low non-specific binding and high S/N ratios. Figure 12 illustrates the use of the modified beads (FB-beads) to immobilize both fluorescein-labeled IgG and IgG that has been double-labeled (fluorescein and HyNic). Specific results are discussed in the figure caption.



A: IgG-F* + amino-beads
B: IgG-F*/HyNic (50µg) + FB-beads
C: IgG-F*/HyNic (100µg) + FB-beads
D: IgG-F*/HyNic (150µg) + FB-beads
E: IgG-F* + FB-beads

FIGURE 12 • Amino beads (A) were incubated with (non-HyNic) fluorescein-labeled IgG (IgG-F*), FB-beads (A,B, C, and D) were incubated with 50, 100, and 150ug IgG-F* modified with HyNic, respectively. Another negative control (non-HyNic modified IgG-F*) was also incubated with FB-beads (E). The beads were then analyzed by FACS. Results demonstrate that there is significant non-specific binding or absorption of IgG-F* with standard amino-beads as seen in Panel (A) but very low background binding of unmodified IgG-F* with FB-beads as seen in Panel (E). Panel's B-D demonstrate a gradual increase in average signal derived from HyNic-modified IgG-F* incubated with FB-beads. As an increase in the amount of added HyNic-modified protein is added the average S/N ratio between Panel D and Panel E (true negative control) is approximately 1000:1.

Additional Facts about SoluLinK's Conjugation Chemistry

Why is SoluLinK's bis-Aryl Hydrazone Bond Stable without a Reduction Step?

There are many references citing the use of Schiff's bases in the formation of conjugates[3]. In most cases, a reduction step is required to form a stable linkage as shown in Figure 13.

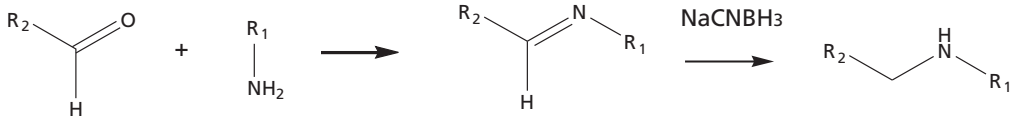


FIGURE 13 • Schiff base formation and subsequent reduction with sodium cyanoborohydride to form a stable bond.

In contrast, SoluLinK's Schiff base is formed from an aromatic hydrazine and an aromatic aldehyde yielding the first example of a bis-aryl hydrazone that is stable under the following conditions:

- thermostable at temperatures ≥90°C for two hours
- stable to hydrolysis in the pH range 2–11 @ RT for 16 hr

There are two factors that make this hydrazone bond so stable. First it uses a hydrazine moiety and not a hydrazide moiety, secondly it forms a bis-aryl hydrazone bond that further stabilizes the hydrazone by resonance contribution from the nearby aromatic constituents. A basic understanding of the mechanism for hydrolysis of Schiff's bases leads to an understanding of why the bis-aryl hydrazone bond is so uniquely stable. Figure 14

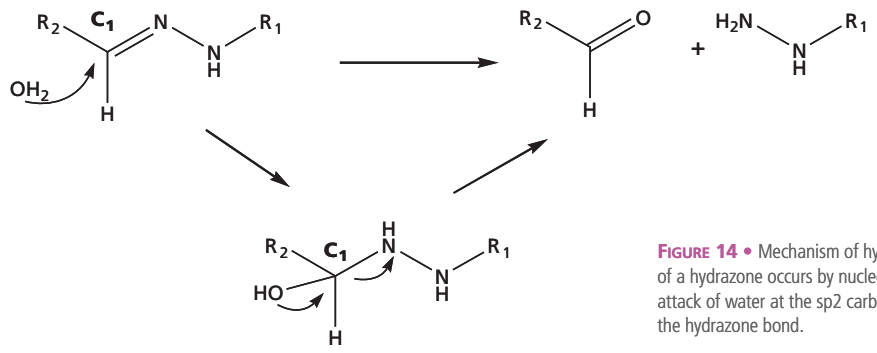


FIGURE 14 • Mechanism of hydrolysis of a hydrazone occurs by nucleophilic attack of water at the sp2 carbon of the hydrazone bond.

Conjugation Background

presents the mechanism of Schiff base hydrolysis. The rate of hydrolysis is affected by pH and temperature of the reaction as well as by the electron density at the C_1 carbon of the hydrazone. The greater the electron density at this carbon the more stable the hydrazone is to hydrolysis (Figure 15).

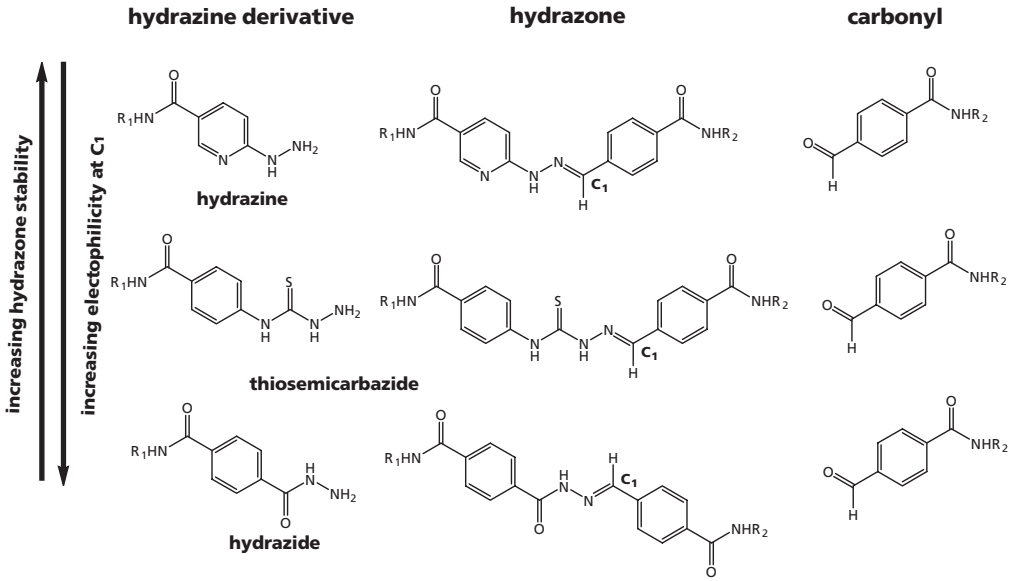


FIGURE 15 • Hydrazone bond stability is inversely related to the increasing electrophilicity at the C_1 carbon as the above compounds progress from the hydrazide to the hydrazine.

References

1. Braslawsky *et al.*, *Cancer Control*, 2002
2. Braslawsky *et al.*, *Cancer Immunology, Immunotherapy,* 2003
3. T. Kaneko *et al*., *Bioconjug Chem.*, 1991 133-41.
4. R. Greenfield *et al*., *Cancer Res.* 1990 6600-7.
5. T Kiessiq, Prstmann T, Enzyme immunoassay techniques. *J Immunol Methods,* 1992, 150, 5-21 (see for example, T.P. King, S.W. Zhao and T. Lam, *Biochemistry.* 1986 25, 5774-9.
6. Bioconjugate Techniques and G. Hermanson, Academic Press.The Bioconjugation Process

Conjugation Kits

Features & Benefits of the SoluLinK Bioconjugation Kits

The chemistry offered in the SoluLinK Bioconjugation Kit is superior to conventional methods of bioconjugation such as maleimide/thiol and avidin/biotin. Using a highly selective interaction of hydrazine and carbonyl groups, the coupling reaction results in a hydrazone bond that is extremely stable. Additionally, no reducing reagents are required, removing the risk of protein denaturing and loss of activity.

Rapid, simple protocol — SoluLinK Bioconjugation enables easy formation of conjugates in ~4 hours.

Specific — The conjugation reaction between the crosslinkers is highly specific. Non-specific binding is virtually eliminated.

No cross linking — SoluLinK bioconjugation chemistry does not crosslink proteins via exposed cysteine residues. Polymerization and self-conjugation does not occur.

Stable conjugates — Conjugates are stable for months, allowing activation in advance and storage for future conjugations.

S-HyNic (SANH) Bioconjugation Kit

Product Description: S-HyNic Bioconjugation Kit provides a powerful yet simple technology for labeling peptides, proteins, carbohydrates and nucleic acids. The kit provides the heterobifunctional linkers S-4FB and S-HyNic along with reagents for quantifying conjugation efficiencies. It enables conjugation of various tags including enzymes, fluorophores, biotin and other tags with a variety of proteins.

Kit Contents

Product	Description	Catalog #	Size/Quantity	Price
S-HyNic (SANH) Kit		S-9002-2	Kit	$ 495
Kit Components	S-HyNic (SANH)		10 mg	
	S-4FB (SFB)		10 mg	
	4-nitrobenzaldehyde		100 mg	
	2-hydrazinopyridine.2 HCL		100 mg	
	2-sulfobenzaldehyde		100 mg	
	DMF		1.0 mL	
	10x Modification Buffer		1.5 mL	
	10x Conjugation Buffer,		1.5 mL	
	Diafiltration Apparatus (5K MWCO)		5 per kit	
Related Products	S-HyNic (SANH) and S-4FB (SFB) linkers are offered separately in several sizes to accommodate different sample sizes.			
	S-HyNic (SANH) Linker	S-1002-105	5 x 1.0 mgs	
	S-HyNic (SANH) Linker	S-1002-010	10 mgs	
	S-4FB (SFB) Linker	S-1004-105	5 x 1.0 mgs	
	S-4FB (SFB) Linker	S-1004-025	25 mgs	

Conjugation Kits

SHTH Conjugation Kit

Product Description: SHTH Bioconjugation Kit provides a powerful yet simple technology for labeling peptides, proteins, carbohydrates, and nucleic acids. The kit provides the heterobifunctional linkers S-4FB and SHTH along with reagents for quantifying fluorophores, biotin and other tags with a variety of proteins.

Kit Contents

Product	Description	Catalog #	Size/Quantity	Price
SHTH Kit		S-9003-1	Kit	$ 425
Kit Components	S-HyNic (SANH)		10 mg	
	S-4FB (SFB)		10 mg	
	4-nitrobenzaldehyde		100 mg	
	2-hydrazinopyridine.2 HCL		100 mg	
	2-sulfobenzaldehyde		100 mg	
	DMF		1.0 mL	
	10x Modification Buffer		1.5 mL	
	10x Conjugation Buffer		1.5 mL	
	Diafiltration Apparatus (5K MWCO)		5 per kit	
Related Products	SHTH and S-4FB (SFB) linkers are offered separately in several sizes to accommodate different sample sizes.			
	SHTH Linker	S-1003-105	5 x 1.0 mg	
	SHTH Linker	S-1003-010	10 mg	
	S-4FB (SFB) Linker	S-1004-105	5x 1.0 mg	
	S-4FB (SFB) Linker	S-1004-025	25 mg	

“We have successfully used the versatile linker, S-HyNic (SANH) (Succinimidyl 6-hydrazinonicotinate acetone hydrazone), to modify proteins of interest, namely mouse serum albumin and ovalbumin, and subsequently conjugate the intermediate to a small molecule containing an aromatic aldehyde to form a stable hydrazone product. The reactions were found to be reproducible and convenient to perform. The final products were easily characterized by UV spectrophotometry to determine the extent of conjugation. S-HyNic is the most versatile and convenient linker reagent we have found for our protein-small molecule modifications.”

Conjugation Kits

SHNH ^{99M}Tc Technicium Conjugation Kit

Product Description: SHNH Bioconjugation Kit provides a powerful yet simple technology for labeling with technicium. The kit provides the heterobifunctional linkers S-4FB and SHNH along with reagents for quantifying conjugation efficiencies. It enables conjugation of various tags including enzymes, fluorophores, biotin and other tags with a variety of proteins.

Product	Description	Catalog #	Size/Quantity	Price
SHNH ^{99M}Tc Kit		S-9003-2	Kit	$ 425
Kit Components	SHNH ^{99M}Tc		10 mg	
	4-nitrobenzaldehyde		100 mg	
	DMF		1.0 mL	
	10x Modification Buffer		1.5 mL	
	Diafiltration Apparatus (5K MWCO)		5 per kit	
Related Products	SHNH ^{99M}Tcand S-4FB (SFB) linkers are offered separately in several sizes to accommodate different sample sizes.			
	SHNH ^{99M}Tc Linker	S-1003-105	5 x 1.0 mg	
	SHNH ^{99M}Tc Linker	S-1003-010	10 mg	

Conjugation Linker Reagents

All of SoluLinK's conjugation linker reagents share a robust feature set resulting in significant benefits:

- **Stable:** forms a covalent hydrazone bond (94°C 2 hr)
- **Traceable:** spectral confirmation of conjugate formation and purification
- **Conjugation Control:** molar substitution ratio (MSR) readily determined
- **Biocompatible:** reducing agents not required, *e.g.*, cyanoborohydride
- **Specificity:** reacts only with aromatic aldehydes in the presence of $–NH_2$, –SH, –COOH and other protein functionalities

The conjugation process provides the benefit of a standard for all linker reagents:

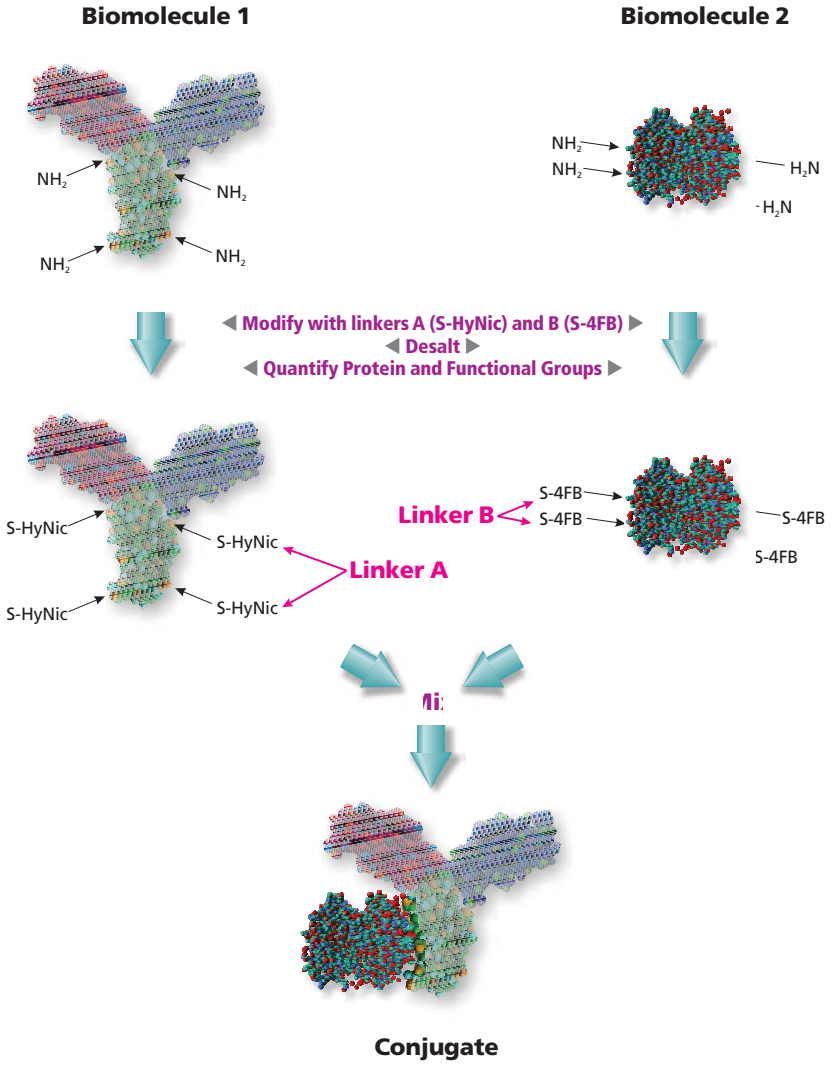
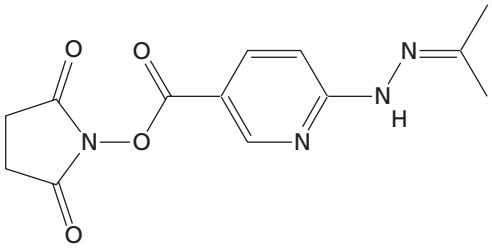


FIGURE 1 • Diagram illustrating the SoluLinK conjugation process with HyNic and SFB.

Conjugation Linker Reagents

S-HyNic (SANH)

(Succinimidyl 6-hydrazinonicotinate acetone hydrazone)



$C_{13}H_{14}N_4O_4$ — MW 290.27 — pale yellow solid

Product Description: S-HyNic is SoluLinK's most popular conjugation reagent, especially for proteins. Its advantages include reaction specificity, UV-traceability, and the unique control it brings to the entire conjugation process. S-HyNic readily reacts with primary amines on a protein (ε-amino group of lysine) via an NHS-ester. S-HyNic introduces traceable HyNic groups (hydrazinonicotinate) into proteins or other biomolecules. HyNic-modified proteins then react to form stable conjugates in the presence of other (aromatic) aldehyde-modified proteins or biomolecules.

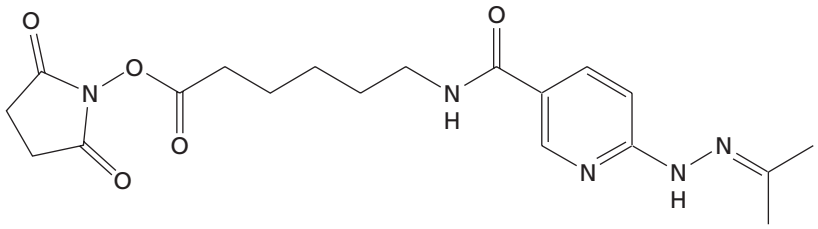
Applications: S-HyNic is most widely used to produce a variety of antibody-related conjugates such as IgG-HRP, IgG-AP and IgG-oligo. Conjugates of other antibody fragments have also been made [scFv, Fab, and F (ab')]. S-HyNic is also used for the synthesis of numerous other conjugates including: general protein-protein, protein-peptide, peptide-oligonucleotide, enzyme-oligonucleotide, and DNA or RNA-protein conjugates. Bioconjugates made using S-HyNic can be used in ELISA assays, Immuno-PCR, *in situ* detection (FISH), Westerns, Southerns, and in many other biological applications.

Product	Description	Catalog #	Size/Quantity	Price
S-HyNic (SANH)	Reagent	S-1002-105	5 x 1.0 mg	$ 150
	Reagent	S-1002-010	10 mg	$ 225
Other Materials Required	S-HyNic (SANH) is used in conjunction with aldehyde modification reagents			
	S-4FB (SFB)	S-1004-025	25 mg	
	10x Modification Buffer	S-4003-015	1.5 mL	
	10x Conjugation Buffer	S-4002-015	1.5 mL	
	Anhydrous DMF	S-4001-010	1.0 mL	
Related Products	S-HyNic (SANH) Complete Kit	S-9002-1		
	SHNH	S-1001-010		
	SHTH	S-1003-010		
	MHPH	S-1002-010		
Protocol	www.solulink.com/protocols/S-HyNic (SANH)			
Product Data Sheet	www.solulink.com/productdatasheet/S-HyNic (SANH)			
MSDS	www.solulink.com/msds/S-HyNic (SANH)			

Conjugation Linker Reagents

C6-S-HyNic (SANH)

(C6-Succinimidyl 6-hydrazinonicotinate acetone hydrazone)



$C_{19}H_{25}N_5O_5$ — MW 403.43 — yellow solid

Product Description: C6-S-HyNic has a six carbon chain added to S-HyNic, SoluLinK's most popular conjugation reagent. Why would you need the six carbon linker? Used to convert primary amines to HyNic (6-hydrazinonicotinamide) moieties with an extended C-6 alkyl linker where protection of the hydrazine is required. The protecting group leaves during formation of the hydrazone conjugate in aqueous media. The extended linker is useful when steric blocking interferes with conjugations especially in the conjugation of two proteins.

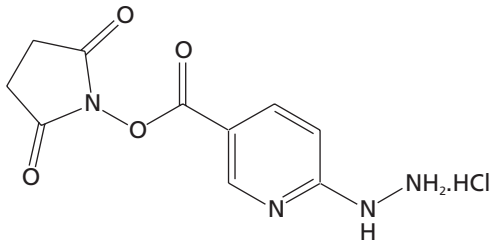
Applications: C6-S-HyNic can be used for the synthesis of numerous conjugates including: general protein-protein, protein-peptide, peptide-oligonucleotide, enzyme-oligonucleotide, and DNA or RNA-protein conjugates. Bioconjugates made using C6-S-HyNic can be used in ELISA assays, Immuno-PCR, *in situ* detection (FISH), Westerns, Southerns, and in many other biological applications.

Product	Description	Catalog #	Size/Quantity	Price
C6-S-HyNic (SANH)	Reagent	S-1010-105	5 x 1.0 mg	$ 175
	Reagent	S-1010-010	10 mg	$ 250
Other Materials Required	C6-S-HyNic (SANH) is used in conjunction with aldehyde modification reagents			
	S-4FB (SFB)	S-1004-025	25 mg	
	10x Modification Buffer	S-4003-015	1.5 mL	
	10x Conjugation Buffer	S-4002-015	1.5 mL	
	Anhydrous DMF	S-4001-010	1.0 mL	
Related Products	S-HyNic (SANH) Complete Kit	S-9002-1		
	SHNH	S-1001-010		
	SHTH	S-1003-010		
	MHPH	S-1002-010		
Protocol	www.solulink.com/protocols/C6SANH			
Product Data Sheet	www.solulink.com/productdatasheet/C6SANH			
MSDS	www.solulink.com/msds/c6SANH			

Conjugation Linker Reagents

SHNH

(Succinimidyl hydraziniumnicotinate)



$C_{10}H_{10}N_4O_4HCl$ — MW 286.68 — white solid

Product Description: SHNH readily reacts with primary amines on a protein (ε-amino group of lysine) via an NHS-ester. SHNH introduces traceable HyNic groups (hydrazinonicotinate) into proteins or other biomolecules. HyNic-modified proteins then react to form stable conjugates in the presence of other (aromatic) aldehyde-modified proteins or biomolecules.

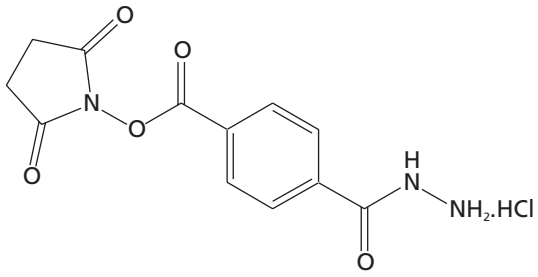
Applications: SHNH was developed for the conjugation of technicium for medical imaging and other applications.

Product	Description	Catalog #	Size/Quantity	Price
SHNH	Reagent	S-1001-105	5 x 1.0 mg	$ 175
	Reagent	S-1001-010	10 mg	$ 250
Other Materials Required	SHNH is used in conjunction with aldehyde modification reagents			
	S-4FB (SFB)	S-1004-025	25 mg	
	10x Modification Buffer	S-4003-015	1.5 mL	
	10x Conjugation Buffer	S-4002-015	1.5 mL	
	Anhydrous DMF	S-4001-010	1.0 mL	
Related Products	SHNH Complete Kit	S-9002-3		
	S-HyNic (SANH)	S-1002-010		
	SHTH	S-1003-010		
	MHPH	S-1002-010		
Protocol	www.solulink.com/protocols/SHNH			
Product Data Sheet	www.solulink.com/productdatasheet/SHNH			
MSDS	www.solulink.com/msds/shnh			

Conjugation Linker Reagents

SHTH

(Succinimidyl 4-hydrazidoterephthalate.hydrochloride)



$C_{12}H_{11}N_3O_5HCl$ — MW 313.69 — white solid

PRODUCT DESCRIPTION: This reagent is used to convert primary amines on biomolecules and surfaces to 4-hydrazidobenzamide moieties in a single step. Its advantages include reaction specificity, UV-traceability, and the unique control it brings to the entire conjugation process. SHTH readily reacts with primary amines on a protein (ε-amino group of lysine) via an NHS-ester. SHTH introduces traceable HyNic groups (hydrazinonicotinate) into proteins or other biomolecules. HyNic-modified proteins then react to form stable conjugates in the presence of other (aromatic) aldehyde-modified proteins or biomolecules.

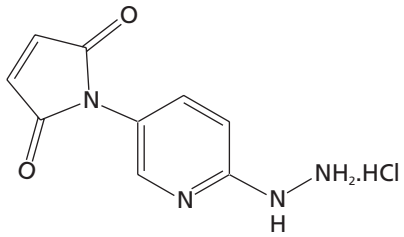
APPLICATIONS: SHTH is most widely used to produce a variety of antibody-related conjugates such as IgG-HRP, IgG-AP and IgG-oligo. Conjugates of other antibody fragments have also been made [scFv, Fab, and F(ab')]. SHTH is also used for the synthesis of numerous other conjugates including general protein-protein, protein-peptide, peptide-oligonucleotide, enzyme-oligonucleotide, and DNA or RNA-protein conjugates. Bioconjugates made using SHTH can be used in ELISA assays, Immuno-PCR, *in situ* detection (FISH), Westerns, Southerns, and in many other biological applications.

Product	Description	Catalog #	Size/Quantity	Price
SHTH	Reagent	S-1003-105	5 x 1.0 mg	$ 175
	Reagent	S-1003-010	10 mg	$ 225
Other Materials Required	SHTH is used in conjunction with aldehyde modification reagents			
	S-4FB (SFB)	S-1004-025	25 mg	
	10x Modification Buffer	S-4003-015	1.5 mL	
	10x Conjugation Buffer	S-4002-015	1.5 mL	
	Anhydrous DMF	S-4001-010	1.0 mL	
Related Products	SHTH Complete Kit	S-9002-1		
	S-HyNic (SANH)	S-1002-010		
	SHNH	S-1001-010		
	MHPH	S-1002-010		
Protocol	www.solulink.com/protocols/SHTH			
Product Data Sheet	www.solulink.com/productdatasheet/SHTH			
MSDS	www.solulink.com/msds/shth			

Conjugation Linker Reagents

MHPH

(3-N-Maleimido-6-hydraziniumpyridine hydrochloride)



$C_9H_8N_4O_2$ — MW 240.65 — yellow solid

Product Description: This reagent is used to convert thiol moieties on biomolecules and surfaces to 2-hydrazinopyridine moieties in a single step. Its advantages include reaction specificity, UV-traceability, and the unique control it brings to the entire conjugation process. MHPH readily reacts with primary amines on a protein (ε-amino group of lysine) via an NHS-ester. MHPH introduces traceable HyNic groups (hydrazinonicotinate) into proteins or other biomolecules. HyNic-modified proteins then react to form stable conjugates in the presence of other (aromatic) aldehyde-modified proteins or biomolecules.

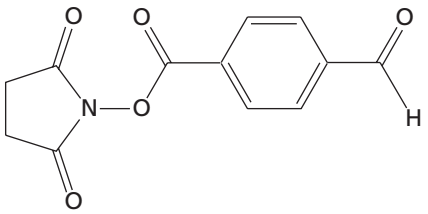
Applications: MHPH is most widely used to produce a variety of antibody-related conjugates such as IgG-HRP, IgG-AP and IgG-oligo. Conjugates of other antibody fragments have also been made [scFv, Fab, and F(ab)']. MHPH is also used for the synthesis of numerous other conjugates including general protein-protein, protein-peptide, peptide-oligonucleotide, enzyme-oligonucleotide, and DNA or RNA-protein conjugates. Bioconjugates made using MHPH can be used in ELISA assays, Immuno-PCR, *in situ* detection (FISH), Westerns, Southerns, and in many other biological applications.

Product	Description	Catalog #	Size/Quantity	Price
MHPH	Reagent	S-1009-105	5 x 1.0 mg	$ 175
	Reagent	S-1009-010	10 mg	$ 250
Other Materials Required	MHPH is used in conjunction with aldehyde modification reagents			
	S-4FB (SFB)	S-1004-025	25 mg	
	10x Modification Buffer	S-4003-015	1.5 mL	
	10x Conjugation Buffer	S-4002-015	1.5 mL	
	Anhydrous DMF	S-4001-010	1.0 mL	
Related Products	MHPH Complete Kit	S-9002-1		
	S-HyNic (SANH)	S-1002-010		
	SHNH	S-1001-010		
	MHPH	S-1002-010		
Protocol	www.solulink.com/protocols/MHPH			
Product Data Sheet	www.solulink.com/productdatasheet/MHPH			
MSDS	www.solulink.com/msds/mhph			

Aldehyde Modification Reagents

S-4FB (SFB)

(Succinimidyl 4-formylbenzoate)



$C_{12}H_9NO_5$ — MW 247.1 — white solid

Product Description: S-4FB is a heterobifunctional linker used to make bioconjugates. This linker reacts with primary amines on biomolecules and converts them to 4-formylbenzamide (4-FB) moieties. Formylbenzamide-modified biomolecules form stable bis-aryl hydrazone conjugates on reaction with HyNic-modified biomolecules. This aromatic aldehyde has the longest reactive stability of any known conjugate forming linker (>1 yr) and makes it the ideal choice for modifying surfaces such as beads. The extended linker is useful when steric blocking interferes with conjugations especially in the conjugation of two proteins.

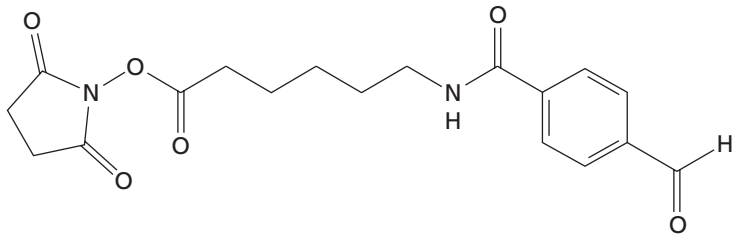
Applications: S-4FB is used to convert amines to benzaldehyde for binding to another biomolecule or to a solid surface that has a SoluLinK linker attached like S-HyNic. S-4FB is also used for the synthesis of numerous other conjugates including: general protein-protein, protein-peptide, peptide-oligonucleotide, enzyme-oligonucleotide, and DNA or RNA-protein conjugates. Bioconjugates made using S-4FB can be used in ELISA assays, Immuno-PCR, *in situ* detection (FISH), Westerns, Southerns, and in many other biological applications.

Product	Description	Catalog #	Size/Quantity	Price
S-4FB (SFB)	Reagent	S-1004-025	25 mg	$ 150
	Reagent	S-1004-105	5 x 1.0 mg	$ 100
Other Materials Required	used in conjunction with the conjugation reagents			
	A linker, *e.g.*, S-HyNic (SANH)	S-1002-025	5 x 1.0 mg	
	10x Modification Buffer	S-4003-015	1.5 mL	
	10x Conjugation Buffer	S-4002-015	1.5 mL	
	Anhydrous DMF	S-4001-010	1.0 mL	
Related Products	S-HyNic (SANH) Complete Kit	S-9002-1		
	sulfo-S-4FB (SFB)	S-1008		
	C6-S-4FB (SFB)	S-1007		
Protocol	www.solulink.com/protocols/S-4FB (SFB)			
Product Data Sheet	www.solulink.com/productdatasheet/S-4FB (SFB)			
MSDS	www.solulink.com/msds/S-4FB (SFB)			

Aldehyde Modification Reagents

C6-S-4FB

(C6-Succinimidyl 4-formylbenzoate)



$C_{18}H_{20}N_2O_6$ — MW 360.4 — white powder

Product Description: C6-S-4FB is a new water-soluble heterobifunctional linker used to make bioconjugates and modify surfaces. This linker reacts with primary amines on biomolecules and converts them to 4-formylbenzamide (4-FB) moieties. Formylbenzamide-modified molecules form stable bis-aryl hydrazone conjugates on reaction with HyNic-modified biomolecules. The increased water-solubility provided by this S-4FB analog makes it particular useful for the modification of beads or other surfaces where high concentrations of linker are required or where use of DMF or DMSO solvents might affect the biological function of the conjugated protein.

Features

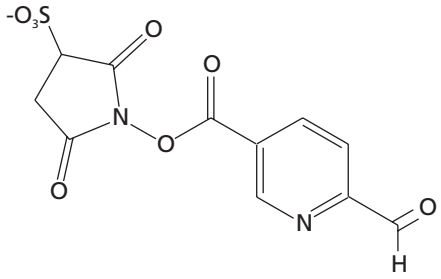
- Heterobifunctional linker used for making bioconjugates
- Water-soluble analog of S-4FB
- Introduction of stable aromatic aldehydes into biomolecules
- Non-cleavable
- Reactive groups: Sulfo-NHS-ester, aromatic aldehyde
- Reactive towards: amino groups, aromatic hydrazines

Product	Description	Catalog #	Size/Quantity	Price
C6-S-4FB (SFB)	Reagent	S-1007-025	25 mg	$ 200
	Reagent	S-1007-105	5 x 1.0 mg	$ 150
Other Materials Required	used in conjunction with the conjugation reagents			
	A linker, *e.g.*, S-HyNic (SANH)	S-1002-025	5 x 1.0 mg	
	10x Modification Buffer	S-4003-015	1.5 mL	
	10x Conjugation Buffer	S-4002-015	1.5 mL	
	Anhydrous DMF	S-4001-010	1.0 mL	
Related Products	S-4FB (SFB)	S-1004		
	PEG4/PFB	S-1034		
	MTFB	S-1035		
Protocol	www.solulink.com/protocols/C6-S-4FB (SFB)			
Product Data Sheet	www.solulink.com/productdatasheet/C6-S-4FB (SFB)			
MSDS	www.solulink.com/msds/C6-S-4FB (SFB)			

Aldehyde Modification Reagents

Sulfo-S-4FB (Sulfo-SFB)

(sulfo-Succinimidyl 4-formylbenzoate)



$C_{12}H_8NO_8S.Na+$ — MW 348.99 — white powder

Product Description: Sulfo-S-4FB is a new water-soluble heterobifunctional linker used to make bioconjugates and modify surfaces. This linker reacts with primary amines on biomolecules and converts them to 4-formylbenzamide (4-FB) moieties. Formylbenzamide-modified molecules form stable bis-aryl hydrazone conjugates on reaction with HyNic-modified biomolecules. The increased water-solubility provided by this S-4FB analog makes it particular useful for the modification of beads or other surfaces where high concentrations of linker are required or where use of DMF or DMSO solvents might affect the biological function of the conjugated protein.

Features

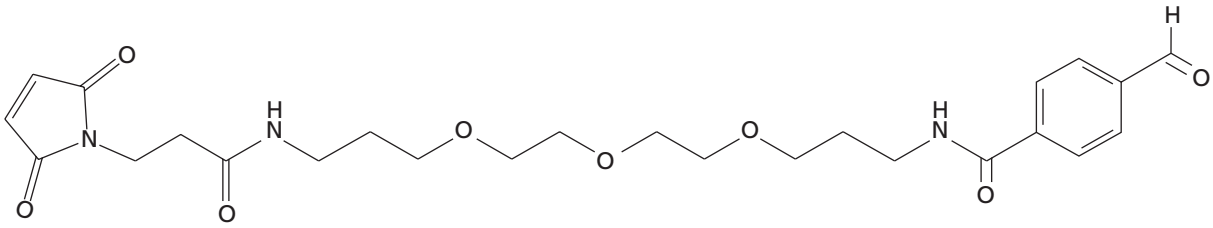
- Heterobifunctional linker used for making bioconjugates
- Water-soluble analog of S-4FB
- Introduction of stable aromatic aldehydes into biomolecules
- Non-cleavable
- Reactive groups: Sulfo-NHS-ester, aromatic aldehyde
- Reactive towards: amino groups, aromatic hydrazines

Product	Description	Catalog #	Size/Quantity	Price
sulfo-S-4FB (SFB)	Reagent	S-1008-025	10 mg	$ 200
	Reagent	S-1008-105	5 x 1.0 mg	$ 150
Other Materials Required	used in conjunction with the conjugation reagents			
	A linker, *e.g.*, S-HyNic (SANH)	S-1002-025	5 x 1.0 mg	
	10x Modification Buffer	S-4003-015	1.5 mL	
	10x Conjugation Buffer	S-4002-015	1.5 mL	
	Anhydrous DMF	S-4001-010	1.0 mL	
Related Products	S-4FB (SFB)	S-9004		
Protocol	www.solulink.com/protocols/sulfo-S-4FB (SFB)			
Product Data Sheet	www.solulink.com/productdatasheet/sulfo-S-4FB (SFB)			
MSDS	www.solulink.com/msds/sulfo-S-4FB (SFB)			

Aldehyde Modification Reagents

MTFB

(3-N-Maleimido-6-hydraziniumpyridine hydrachloride)



$C_{25}H_{33}N_3O_8$ — MW 503.54 — white powder

Product Description: MTFB Application: Used to convert primary amines to benzaldehyde moieties. Especially useful for conversion of amino surfaces such as beads and plates as this reagent is water soluble.

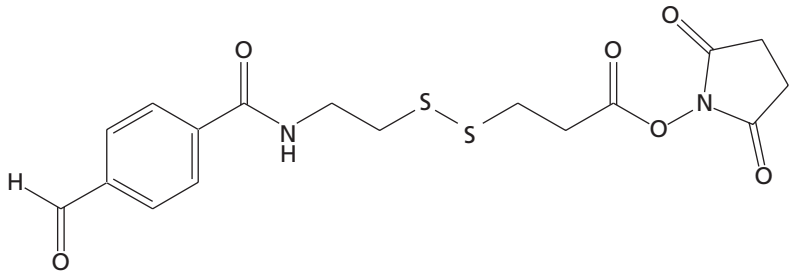
Applications: This reagent is used to convert thiol moieties on biomolecules and surfaces to 4-formylbenzamide (4-FB) moieties in a single step. This molecules possess a PEG3 linker for increased solubility of the modified biomolecule.

Product	Description	Catalog #	Size/Quantity	Price
MTFB	Reagent	S-1035-025	25 mg	$ 150
	Reagent	S-1035-105	5 x 1.0 mg	$ 100
Other Materials Required	used in conjunction with the conjugation reagents			
	A linker, *e.g.*, S-HyNic (SANH)	S-1002-025	5 x 1.0 mg	
	10x Modification Buffer	S-4003-015	1.5 mL	
	10x Conjugation Buffer	S-4002-015	1.5 mL	
	Anhydrous DMF	S-4001-010	1.0 mL	
Related Products	S-4FB (SFB)	S-1004		
	C6-S-4FB	S-1007		
	PEG4/PFB	S-1034		
Protocol	www.solulink.com/protocols/MTFB			
Product Data Sheet	www.solulink.com/productdatasheet/MTFB			
MSDS	www.solulink.com/msds/MTFB			

Aldehyde Modification Reagents

SS-S-4FB (SFB)

(disulfide-Succinimidyl 4-formylbenzoate)



$C_{17}H_{18}N_2O_6S_2$ — MW 410.46 — white powder

PRODUCT DESCRIPTION: SS-S-4FB is a new cleavable heterobifunctional linker use to make bioconjugates. This linker reacts with primary amines on biomolecules and converts them to 4-formylbenzamide (4-FB) moieties. Formylbenzamide-modified molecules spontaneously form stable bis-aryl hydrazone conjugates on reaction with HyNic-modified biomolecules. SS-S-4FB contains a thiol cleavable disulfide linker where intracellular cleavage of the conjugate is required for cytoplasmic release and activity.

Features

- Heterobifunctional linker used for making bioconjugates
- Water-soluble analog of S-4FB (SFB)
- Introduction of stable aromatic aldehydes into biomolecules
- Non-cleavable
- Reactive groups: Sulfo-NHS-ester, aromatic aldehyde
- Reactive towards: amino groups, aromatic hydrazines

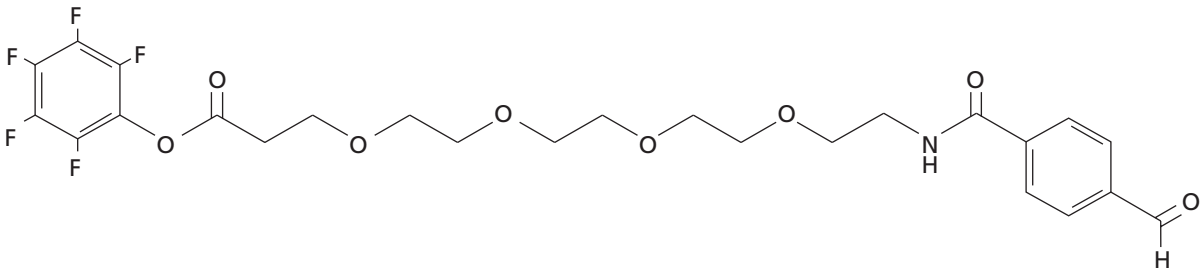
APPLICATIONS: SS-S-4FB is also used for the synthesis of numerous other conjugates including: general protein-protein, protein-peptide, peptide-oligonucleotide, enzyme-oligonucleotide, and DNA or RNA-protein conjugates. Bioconjugates made using SS-S-4FB can be used in ELISA assays, Immuno-PCR, *in situ* detection (FISH), Westerns, Southerns, and in many other biological applications.

Product	Description	Catalog #	Size/Quantity	Price
SS-S-4FB (SFB)	Reagent	S-1037-025	25 mg	$ 200
	Reagent	S-1037-105	5 x 1.0 mg	$ 150
Other Materials Required	used in conjunction with the conjugation reagents			
	A linker, *e.g.*, S-HyNic (SANH)	S-1002-025	5 x 1.0 mg	
	10x Modification Buffer	S-4003-015	1.5 mL	
	10x Conjugation Buffer	S-4002-015	1.5 mL	
	Anhydrous DMF	S-4001-010	1.0 mL	
Related Products	S-4FB (SFB)	S-1004		
Protocol	www.solulink.com/protocols/SS-S-4FB (SFB)			
Product Data Sheet	www.solulink.com/productdatasheet/SS-S-4FB (SFB)			
MSDS	www.solulink.com/msds/SS-S-4FB (SFB)			

Aldehyde Modification Reagents

PEG4/PFB

(Penta Fluorobenzamide Polyetheyleneglycol 4)



$C_{25}H_{26}F_5NO_8$ — MW 563.47 — colorless oil

Product Description: PFB/PEG4 is a new class of heterobifunctional linker used to make bioconjugates. This linker reacts with primary amines on biomolecules and converts them to 4-formylbenzamide (4-FB) moieties with an extended PEG4 linker. PFB/PEG4 modified proteins are more water-soluble (less hydrophobic) than S-4FB-modified proteins which can eliminate precipitation of some proteins. The extended PEG linker enhances solubility and alleviates steric hindrance with increases in conjugate yield.

Features

- Heterobifunctional linker used for making bioconjugates
- Introduction of PEG-modified aromatic aldehydes into biomolecules
- Increases water-solubility of modified proteins
- Reactive groups: pentafluorphenol, protected aromatic hydrazine
- Reactive towards: amino groups, aromatic aldehydes

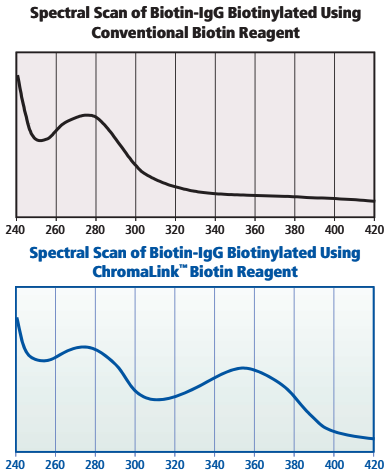
Product	Description	Catalog #	Size/Quantity	Price
PEG4/PFB	Reagent	S-1004-025	25 mg	$ 200
	Reagent	S-1004-105	5 x 1.0 mg	$ 150
Other Materials Required	used in conjunction with the conjugation reagents			
	A linker, *e.g.*, S-HyNic (SANH)	S-1002-025	5 x 1.0 mg	
	10x Modification Buffer	S-4003-015	1.5 mL	
	10x Conjugation Buffer	S-4002-015	1.5 mL	
	Anhydrous DMF	S-4001-010	1.0 mL	
Related Products	S-HyNic (SANH) Complete Kit	S-9002-1		
	S-HyNic (SANH)	S-1002-010		
	SHNH	S-1001-010		
	MHPH	S-1002-010		
Protocol	www.solulink.com/protocols/PEG4/PFB			
Product Data Sheet	www.solulink.com/productdatasheet/PEG4/PFB			
MSDS	www.solulink.com/msds/PEG4/PFB			

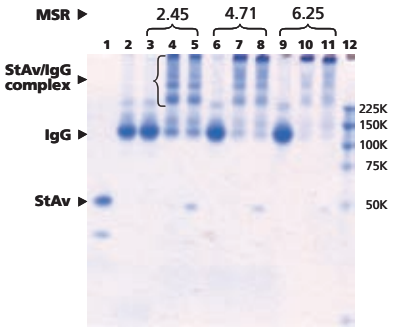
Biotinylation Products & Services

"Our lab does biotinylations on regular basis. The biotinylations went well, but the HABA assay for quantifying biotin was expensive and very time consuming. ChromaLink with its built in quantification signature was far easier and cut down the total time by more than half."

SoluLinK
The Conjugation Company™

Biotinylation Products & Services





Lane	Protein	StAv equiv added
1	Streptavidin	N/A
2	Bovine IgG	N/A
3	IgG-(CL biotin) 2.45	0
4	IgG-(CL biotin) 2.45	1
5	IgG-(CL biotin) 2.45	2
6	IgG-(CL biotin) 4.71	0
7	IgG-(CL biotin) 4.71	1
8	IgG-(CL biotin) 4.71	2
9	IgG-(CL biotin) 6.25	0
10	IgG-(CL biotin) 6.25	1
11	IgG-(CL biotin) 6.25	2
12	MW markers	N/A

FIGURE 2 • Bovine IgG was reacted with 5X, 10X and 15X ChromaLink™ Biotin 354S at 5 mg/mL and desalted into PBS (see above). The protein concentration was determined using the BCA assay and the level of biotin modification was determined spectrophotometrically (extinction coefficient 29,000 at 354 nm). The modified proteins were reacted with 1X and 2X streptavidin and the binding determined by PAGE gel-shift analysis (Coomassie blue staining).

Introducing ChromaLink™ Biotin 354S (Catalog #B-1001-105)

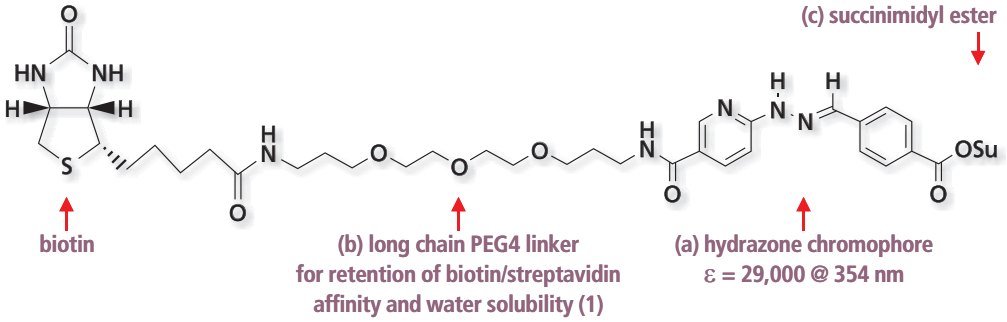
- **Biotinylate & verify using a single reagent**
- **Eliminate the need for the HABA assay**
- **Visualize and quantify biotin incorporation with a simple scan**

Application

ChromaLink Biotin 354S (CLB354S) will incorporate traceable biotins on any amine containing biomolecule or surface. ChromaLink Biotin allows for the simple and direct spectroscopic quantification of total incorporated biotin as illustrated in the protein absorbance spectra.

Technology

ChromaLink Biotin 354S (1) (Catalog #B-1001-105) has been designed to (a) incorporate a bis-aryl hydrazone chromophore (ε = 29,000 @ 354 nm) in the linker that allows for direct spectroscopic quantification of total incorporated biotins, (b) a long chain PEG4 linker to preserve biotin/avidin affinity as well as increase solubility and (c) an aromatic succinimidyl ester that more efficiently modifies amines in aqueous buffers than aliphatic succinimidyl esters.By simply measuring the $Å_{280}$ and $Å_{354}$ of a modified protein the concentration and number of biotins incorporated/protein can be determined directly. Only a 20 µg aliquot of biotin-modified protein in a 100µL volume of buffer is required to yield reproducible results using a standard microplate reader.



Example Application

Procedure to Modify Bovine IgG Using CLB354S

Bovine immunoglobulin was dissolved in modification buffer (100 mM phosphate, 150 mM NaCl, pH 7.2) to prepare a 5 mg/mL solution. A solution of ChromaLink biotin 354S (1) (0.5 mg) dissolved in DMF (50 µL) was prepared. Three separate reactions were performed wherein 5 mol equiv, 10 mol equiv and 15 mol equiv of (1) (1.3, 2.6 and 3.9 µL) respectively were added to 0.5 mg bovine IgG solution. The biotinylation reactions were allowed to incubate at room temperature for 2 hours. The reaction mixtures were desalted into PBS (pH 7.4) using Biomax diafiltration filters (Millipore, Inc., Bedford, MA). Protein concentrations were determined using the BCA assay (Pierce Chemical Co., Rockford, IL). Spectral analyses of each product was performed by diluting 20 µg of modified protein into 100 µL PBS. The number of moles of chromophore incorporated was calculated by determining the

Biotinylation Products & Services

absorbance of the protein at $Å_{354}$ and dividing the value obtained by the molar extinction coefficient of the chromophore (29,000). The overlaid spectra of the products (0.2 µg/µL), as well as unmodified bovine IgG are presented in Figure 1.

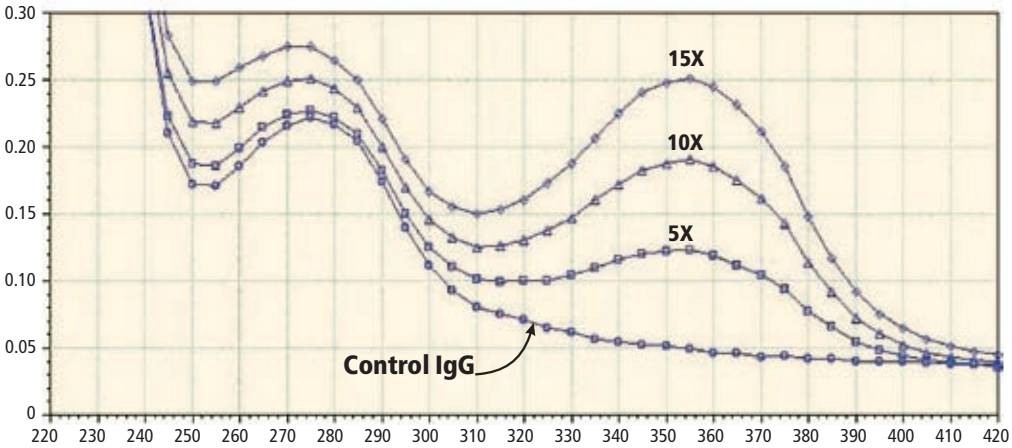


Figure 1 • Superimposed spectra of desalted bovine IgG that was biotinylated using ChromaLink™ Biotin at various biotin to protein mole equivalents (5X, 10X and 15X).

The number of incorporated biotins analyzed using the HABA assay (Pierce Chemical Co., Rockford, IL) are presented in Table 1.

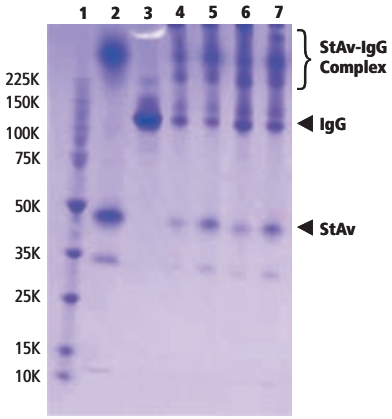
To demonstrate the streptavidin-binding efficiency of ChromaLink biotin, biotinylated bovine IgG was treated with 1 and 2 mole equivalents of streptavidin and binding was confirmed by PAGE gel-shift analysis with the results as shown in Figure 2.

These gel-shift results demonstrate increasing streptavidin binding efficiency with increasing biotin modification. The lack of complete binding at the 2.45 biotins/IgG level is likely due to inaccessibility of some biotins to streptavidin as they may be "hiding" in hydrophobic pockets and reveals the importance of determining the degree of protein biotinylation. In another experiment, ChromaLink Biotin 354S was compared to another commercially available biotinylation reagent for equivalency of streptavidin binding. In these experiments, bovine IgG was labeled with 5X ChromaLink Biotin 354S or 5X biotin-PEG4-succinimidyl ester, respectively. The two sets of biotin-modified IgG were treated with 1 and 2 mole equivalents of streptavidin and binding was once again confirmed by PAGE gel-shift analysis. The PAGE gel and description of the results are presented in Figure 3:

These results demonstrate that IgG modified with ChromaLink Biotin 354S binds to streptavidin identically to IgG modified with biotin/PEG4/succinimidyl ester.

	Biotin/IgG HABA	Biotin/IgG $Å_{354}$
5 x	1.03	2.45
10 x	1.60	4.71
15 x	2.22	6.25

Table 1 • Tabular results illustrating molar substitution ratio (MSR) obtained using two different biotin incorporation methods (ChromaLink™ UV-spectrophotometric assay (354 nm) vs. the HABA assay).



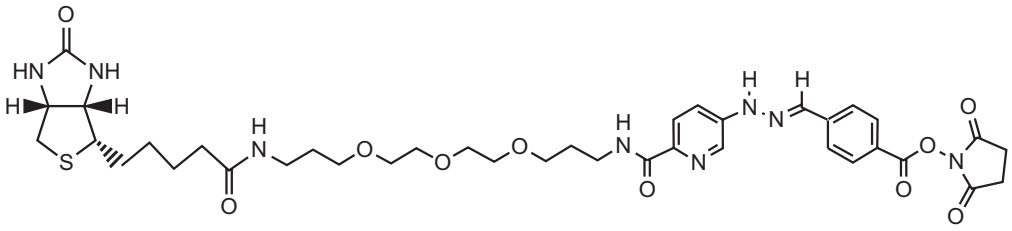
Lane	Protein added	# equiv StAv
1	M.W. Markers	N/A
2	Streptavidin	N/A
3	IgG	N/A
4	IgG-(CL biotin) 2.0 MSR	1.0
5	IgG-(CL biotin) 2.0 MSR	2.0
6	IgG-(PEG4/ biotin) 2.0 MSR	1.0
7	IgG-(PEG4/ biotin) 2.0 MSR	2.0

Figure 3 • Bovine IgG was reacted with 5X ChromaLink Biotin 354 and 5X biotin/PEG4/ succinimidyl ester at 5 mg/mL, and desalted into PBS (see above). The protein concentration was determined using the BCA assay and the level of modification was determined spectrophotometrically (extinction coefficient 29,000 at 354 nm). The modified proteins were reacted with 1X and 2X streptavidin equivalents and the binding determined by PAGE gel-shift analysis (Coomassie blue stain).

Biotinylation Products & Services

ChromaLink™ Biotin (Catalog #B-1001-105)

Biotinylation Reagent with Quantification!



$C_{38}H_{50}N_8O_{10}S$ — MW 810.92 — yellow solid

Application: Incorporation of biotin with a linker that possesses a chromaphore on amino-containing biomolecules and surfaces that allows quantification of biotin by spectroscopic means.

Product	Description	Catalog #	Size/Quantity	Price
ChromaLink Biotin 354S	New Trial Size	B1001-102T	2 x 1.0 mg Kit	$ 99
		B1001-105	5 x 1.0 mg	$ 165
		B1001-110	1 x 10 mg	$ 195
		B1001-105K	5 x 1.0 mg Kit	$ 250
B1001-105K Kit Components	ChromaLink Biotin 354S	B1001-105	5 x 1.0 mg	
	DMF (anhydrous)		1.0 mL	
	10X Modification Buffer		1.5 mL	
	Diafiltration Apparatus (5K MWCO)		4	

Protocol for Incorporation of ChromaLink™ Biotin 354S

1. Exchange protein into 100 mM phosphate, 150 mM NaCl, pH 7.2–7.4 buffer at 2–5 mg/mL
2. Dissolve ChromaLink Biotin 354S (1) (0.5 mg) in anhydrous DMF (50 µL).
3. Add an aliquot containing 10–15 mole equivalents of ChromaLink Biotin 354S (1) to protein solution
4. Incubate at room temperature for 2.0 hr
5. Desalt by dialysis, diafiltraton, or a desalting column into desired buffer
6. Determine the molar substitution ratio (MSR) of biotin on the protein by one of two methods (calculators for both protocols can be found at www.solulink.com/technology.

Biotinylation Products & Services

Biotin/Protein Molar Substitution Ratio (MSR) Calculations

Based on Corrected Absorbance

Determine the $Å_{280}$ and $Å_{354}$ of the modified protein and use the following equations.

1. Determine corrected absorbance ($Åc_{280}$)

Eq 1a $$Åc_{280} = Å_{280} - (Å_{354} \times 0.23)$$

2. Determine moles protein

Note: *protein "A" value for antibodies is 1.2. Volume in mL and protein in mg/mL*

Eq 2a $$\left[\frac{(Åc_{280} \div \textit{protein 'A' value}) \times (\textit{volume})}{1000}\right] \div \textit{protein MW} = \textit{moles protein}$$

3. Determine moles biotin present (volume)

Eq 3a $$\left(\frac{Å_{354}}{29000}\right) \times \left(\frac{\textit{volume}}{1000}\right) = \textit{moles biotin}$$

4. Biotin/protein MSR determination

$$\text{MSR} = \frac{\textbf{Eq 3a}}{\textbf{Eq 2a}} = \frac{\textit{moles biotin}}{\textit{moles protein}}$$

Based on Protein Concentration

Determine the protein concentration using a protein assay such as the BCA or Bradford and determine the $Å_{354}$ and use the following equation

1. Determine moles protein

Note: *volume in mL and protein in mg/mL*

Eq 1b $$\left[\frac{(\textit{protein concentration}) \times (\textit{volume})}{1000}\right] \div \textit{protein MW} = \textit{moles protein}$$

2. Determine moles biotin present:

Eq 2b $$\left(\frac{Å_{354}}{29000}\right) \times \left(\frac{\textit{volume}}{1000}\right) = \textit{moles biotin}$$

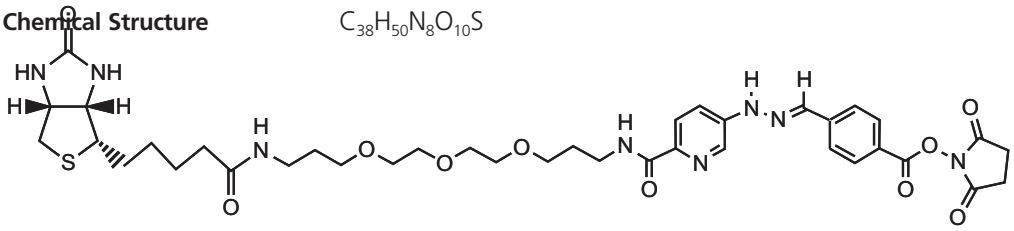
3. Biotin/protein MSR determination

$$\text{MSR} = \frac{\textbf{Eq 2b}}{\textbf{Eq 1b}} = \frac{\textit{moles biotin}}{\textit{moles protein}}$$

Biotinylation Products & Services

ChromaLink Biotin 354S

Chemical Structure $C_{38}H_{50}N_8O_{10}S$

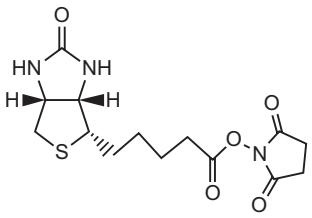


Molecular Weight	810.92
Catalog #	B-1001
Components	ChromaLink Biotin 354S
Application	ChromaLink Biotin 354S (CLB 354S) incorporates biotin on amine containing biomolecules and allows for direct spectrophotometric quantifiation of total biotin.
Comments	Store under inert atmosphere desiccated. Not for internal or external use in humans.
Product Use Limitiations	The products offered here are for research use only.
Licensing	Any commercial application will require a license from SoluLinK. The SoluLinK Conjugation System is patented and has additional patents pending. Please contact SoluLinK for information regarding licensing information. No license is granted or implied to any patents to technologies for which the end user applies our products.

Biotinylation Products & Services

Standard Biotin Reagents

Biotin succinimidyl ester

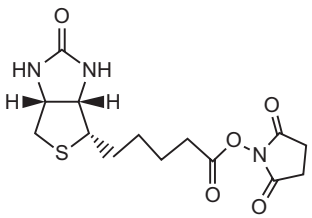


$C_{14}H_{19}N_3O_5S$ — MW 341.38 — white solid

Application: Incorporation of biotin on amine-reactive biomolecules.

Product	Description	Catalog #	Size/Quantity	Price
Biotin succinimidyl ester		B1005-120	10 x 2.0 mg	$ 195
		B1005-100	50 mg	$ 295
		B1005-110K	5 x 2.0 mg Kit	$ 225
B1005-110K Kit Components	Biotin succinimidyl ester		5 x 2.0 mg	
	DMF (anhydrous)		1.0 mL	
	10X Modification Buffer		1.5 mL	
	Diafiltration Apparatus (5K MWCO)		4	

Biotin/PEG4/succinimidyl ester



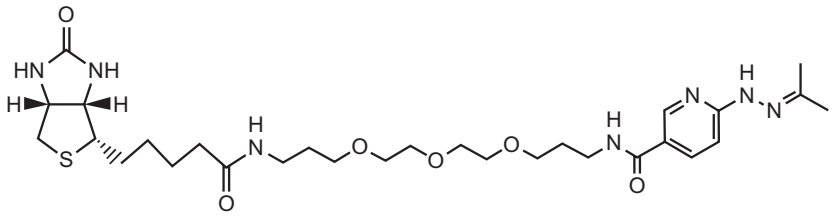
$C_{25}H_{40}N_4O_{10}S$ — MW 588.67 — white solid

Application: Incorporation of biotin with a PEG4 linker on amino-containing biomolecules.

Product	Description	Catalog #	Size/Quantity	Price
Biotin/PEG4/succinimidyl ester		B1002-120	10 x 2.0 mg	$ 195
		B1002-025	25 mg	$ 295
		B1002-110K	5 x 2.0 mg Kit	$ 225
B1002-110K Kit Components	Biotin/PEG4/succinimidyl ester		5 x 2.0 mg	
	DMF (anhydrous)		1.0 mL	
	10X Modification Buffer		1.5 mL	
	Diafiltration Apparatus (5K MWCO)		4	

Biotinylation Products & Services

Biotin/PEG4/hydrazino Nicotinamide Acetone Hydrazone

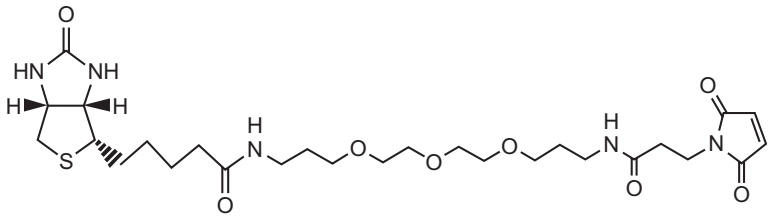


$C_{29}H_{47}N_7O_6S$ — MW 621.79 — yellow solid

Application: Incorporation of biotin with a PEG4 linker on carbonyl containing biomolecules.

Product	Description	Catalog #	Size/Quantity	Price
Biotin/PEG4/hydrazino nicotinamide acetone hydrazone		B1003-105	5 x 1.0 mg	$ 195
		B1003-110K	5 x 2.0 mg Kit	$ 225
B1003-110K Kit Components	Biotin/PEG4/hydrazino nicotinamide acetone hydrazone		5 x 2.0 mg	
	DMF (anhydrous)		1.0 mL	
	10X Modification Buffer		1.5 mL	
	Diafiltration Apparatus (5K MWCO)		4	

Biotin/PEG4/maleimide



$C_{27}H_{43}N_5O_8S$ — MW 597.73 — white solid

Application: Incorporation of biotin with a PEG4 linker on sulfhydryl-containing biomolecules.

Product	Description	Catalog #	Size/Quantity	Price
Biotin/PEG4/maleimide		B1004-120	10 x 2.0 mg	$ 195
		B1004-025	25 mg	$ 295
		B1004-110K	5 x 2.0 mg Kit	$ 225
B1004-110K Kit Components	Biotin/PEG4/maleimide		5 x 2.0 mg	
	DMF (anhydrous)		1.0 mL	
	10X Modification Buffer		1.5 mL	
	Diafiltration Apparatus (5K MWCO)		4	

Detection Kits & Defined Conjugates

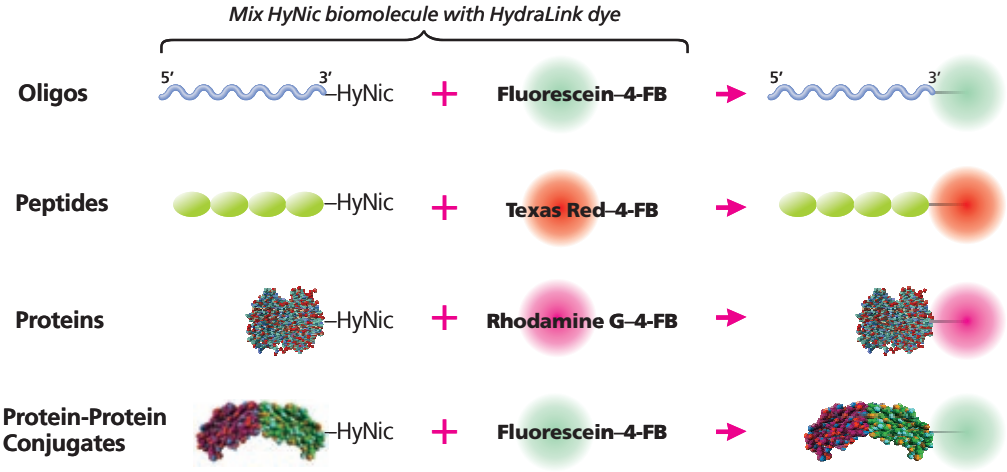
"Conjugating fluors to antibodies was time conuming, so I often sent out to have it done which also took time. Having the "pre-activated" fluors available from SoluLinK made doing the conjugations in-house much more timely and convenient."

SoluLinK
The Conjugation Company™

Detection Kits & Defined Conjugates

HydraLink™ Fluorescent Dyes

SoluLink is now offering three of the most popular fluorescent dyes with HydraLink chemistry for the labeling of proteins, antibodies, oligonucleotides and peptides. Texas Red, Fluorescein, and Rhodamine G fluorescent are available pre-activated with an aromatic aldehyde group (4-FB). These stable pre-activated dyes react and label any HyNic-modified biomolecule. The dyes are especially suited for the fluorescent labeling of



HyNic-modified peptides, *e.g.*, PepLink™ peptides, since HydraLink chemistry does not react with either lysine or cysteine amino acid side chains internal to the peptide. Fluorescently labeled peptides can be used to monitor in vivo internalization, spatial distribution and/or localization. These dyes can also be used to fluorescently label protein-protein or protein-peptide conjugates containing excess HyNic groups on their surface.

Texas Red-FB

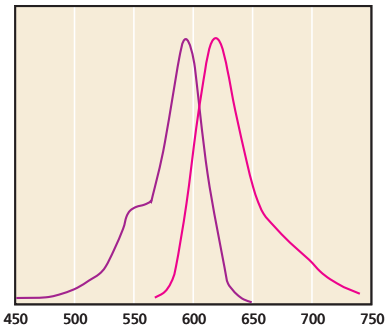
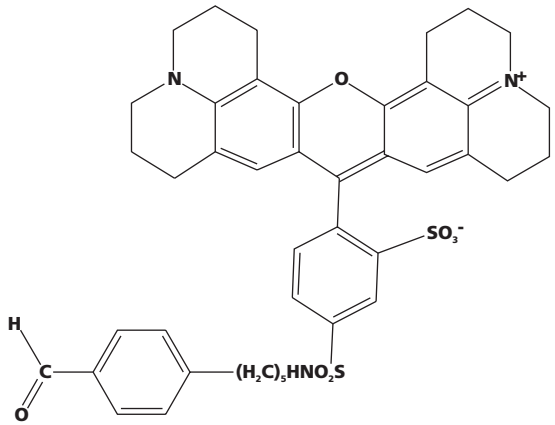


Figure 1 • Absorption and emission spectra of SoluLinK's Texas Red-FB

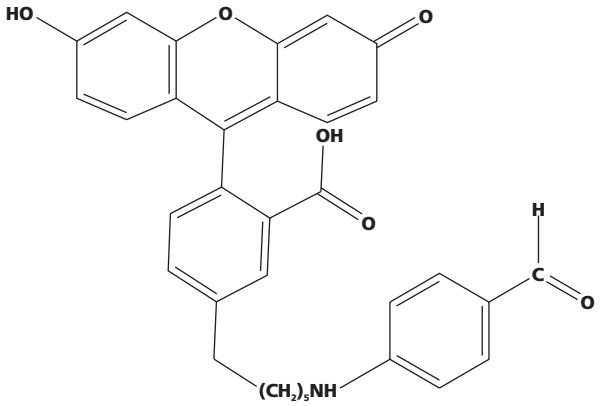


Product Description: Texas Red-FB is an aromatic-aldehyde (formyl benzaminde) modified Texas Red fluorophore that is readily conjugated to any S-HyNic-modified antibody or protein. Texas Red-FB, unlike NHS-esters of the same fluorophore is not susceptible to hydrolysis during reaction with S-HyNic-modified proteins. Efficient labeling of S-HyNic-modified proteins with Fluorescein-FB has the advantage that it does not require desalting of the protein prior to labeling to remove amine-containing contaminants and buf-

Detection Kits & Defined Conjugates

fers. SoluLinK's efficient hydrazine-aldehyde conjugation chemistry permits labeling at low dye-to-protein reaction ratios that form low protein-to-dye substitution ratios. This labeling efficiency guarantees greater control over the labeling reaction and reduces non-specific adsorption of fluorescent conjugates often seen with less controlled (higher) dye to protein substitution ratios. The absorption and emission spectra of Texas Red-FB is illustrated in Figure 1 [aqueous buffer (pH 7)]. Ex/Em:588/625

Fluorescein-FB



Product Description: 4FB-fluorescein is an aromatic-aldehyde (formyl benzaminde) modified 4FB-fluorescein fluorophore that is readily conjugated to any S-HyNic-modified antibody or protein. 4FB-fluorescein, unlike NHS-esters of the same fluorophore is not susceptible to hydrolysis during reaction with S-HyNic-modified proteins. Efficient labeling of S-HyNic-modified proteins with 4FB-fluorescein has the advantage that it does not require desalting of the protein prior to labeling to remove amine-containing contaminants and buffers. SoluLinK's efficient hydrazine-aldehyde conjugation chemistry permits labeling at low dye-to-protein reaction ratios that form low protein-to-dye substitution ratios. This labeling efficiency guarantees greater control over the labeling reaction and reduces non-specific adsorption of fluorescent conjugates often seen with less controlled (higher) dye to protein substitution ratios. The absorption and emission spectra of 4FB-fluorescein-FB is illustrated in Figure 2 [aqueous buffer (pH 8)]. Ex/Em:492/515

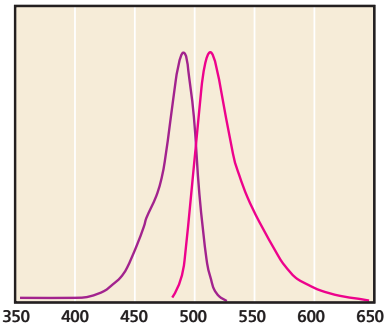
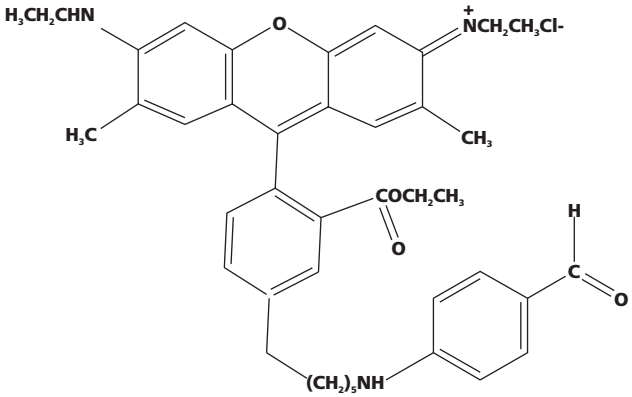


Figure 2 • Absorption and emission spectra of SoluLinK's 4FB-fluorescein.

Detection Kits & Defined Conjugates

Rhodamine G-FB



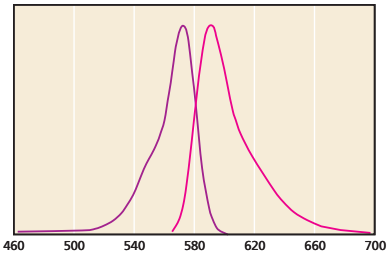


FIGURE 3 • Absorption and emission spectra of SoluLinK's Rhodamine G-FB in aqueous buffer.

PRODUCT DESCRIPTION: Rhodamine G-FB is an aromatic-aldehyde (formyl benzamide) modified Texas Red fluorophore that is readily conjugated to any S-HyNic-modified antibody or protein. Rhodamine G-FB, unlike NHS-esters of the same fluorophore is not susceptible to hydrolysis during reaction with S-HyNic-modified proteins. Efficient labeling of S-HyNic-modified proteins with Fluorescein-FB has the advantage that it does not require desalting of the protein prior to labeling to remove amine-containing contaminants and buffers. SoluLinK's efficient hydrazine-aldehyde conjugation chemistry permits labeling at low dye-to-protein reaction ratios that form low protein-to-dye substitution ratios. This labeling efficiency guarantees greater control over the labeling reaction and reduces non-specific adsorption of fluorescent conjugates often seen with less controlled (higher) dye to protein substitution ratios. The absorption and emission spectra of Rhodamine G-FB is illustrated in Figure 3 [aqueous buffer (pH 7)]. Ex/Em:571/591

Detection Kits & Defined Conjugates

Activated-Biofluorescent Proteins (Phycobiliproteins)

Phycobiliproteins consist of a family of biologically-derived fluorescent proteins found in cyanobacteria and eukaryotic algae. Their intrinsically high fluorescence properties are attributable to covalently-linked tetrapyrrole groups that play a light-collecting role in these organisms. Phycobiliproteins possess highly desirable spectral properties including high molar extinction coefficients and resistance to photobleaching. Phycobiliproteins also exhibit other desirable fluorescent properties including insensitivity to environmental factors such as ionic strength and pH. SoluLinK offers two ready-to-link aromatic aldehyde activated phycobiliproteins.

4FB-Phycoerythrin

Product Description: SoluLinK's 4FB-Phycoerythrin is a formyl benzamide (FB) modified phycobiliprotein. This aldehyde-modified fluorescent protein is ready for linking to any hydrazine-modified protein or biomolecule. Simple mixing of R- Phycoerythrin–FB with any hydrazine-modified protein generates the fluorescent conjugate. Conjugates made using 4FB-Phycoerythrin retain their high quantum yield (~0.98) and are easy to purify using gel filtration methods. They have been used in a variety of applications including microarray gene expression studies (streptavidin-phycoerythrin) and immunostaining applications (antibody-PE conjugates). Other advantages of 4FB-Phycoerythrin include long-term storage stability at 4°C while maintaining its ready-to-link property in aqueous solution for up to 6 months. It also contains a precise number of formyl benzamide groups (quantified with each lot). Quantification provides built-in 'batch to batch' conjugation consistency and performance.

Phycobiliprotein	4FB-Phycoerythrin
MW.	240,000
Absorption Max. Ext.	546,565
Coefficient	2,410,000
Em	575 nm
QY	0.98

4FB-Allophycocyanin

Product Description: SoluLinK's 4FB-Allophycocyanin is a formyl benzamide (FB) modified phycobiliprotein. This aldehyde-modified fluorescent protein is ready for linking to any hydrazine-modified protein or biomolecule. Simple mixing of R-Phycoerythrin–FB with any hydrazine-modified protein generates the fluorescent conjugate. Conjugates made using 4FB-Phycoerythrin retain their high quantum yield (~0.68) and are easy to purify using gel filtration methods. Other advantages to using 4FB-Allophycocyanin include long-term storage stability at 4°C while maintaining its "ready-to-link" property in aqueous solution for up to 6 months. It also contains a precise number of formyl benzamide groups (quantified with each lot). Quantification provides built-in batch-to-batch conjugation consistency and performance.

Phycobiliprotein	4FB-Allophycocyanin
MW	104,000
Absorption Max. Ext.	650
Coefficient	700,000
Em.	650 nm
QY	0.68

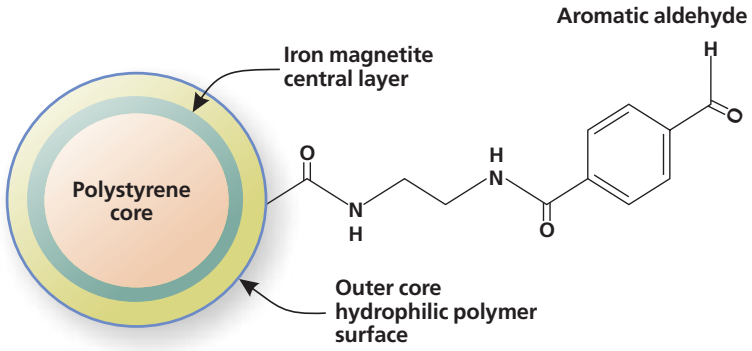
NanoLink™ Magnetic Separation Products

"The Nanolink Magnetic Separation Beads from SoluLinK worked like any other bead we've used — only they had at least 10x the binding capacity — which allowed as to use far fewer beads, plus they remained stable and useful after storage."

SoluLinK
The Conjugation Company™

NanoLink™ Magnetic Separation Products

NanoLink™ S-4FB (SFB) Magnetic Microspheres



PRODUCT DESCRIPTION: NanoLink S-4FB Magnetic particles are uniform, encapsulated, nanometer-sized, super-paramagnetic particles activated with stable aromatic aldehyde functionalities. Aromatic aldehydes are introduced to the hydrophilic amino-modified surface by reaction with SoluLinK's succinimidyl 4-formylbenzoate (S-4FB). The high surface area and low non-specific binding of NanoLink particles are ideal for covalent immobilization of any aromatic hydrazine-modified protein, peptide, DNA, RNA, oligonucleotide and/or oligosaccharide.

Features & Benefits

- High surface area (788 nm ±5% diameter)
- Consistent aromatic aldehyde loading capacities (25–35 nmol/mg)
- Encapsulated (no exposed iron) monodispersed microspheres
- Fast magnetic response time (25 electromagnetic units)
- Stable in collodoidal form
- Stable in detergents
- Paramagnetic (no residual magnetisim)
- Chemically un-reactive with non-hydrazine-modified molecules

The particles are supplied at approximately 1% solids (10 mg/mL) in nuclease-free water with 0.05% sodium azide. There are no surfactants present. Particles can be washed prior to use to remove residual azide, if desired, but it is not necessary for most applications.

Applications

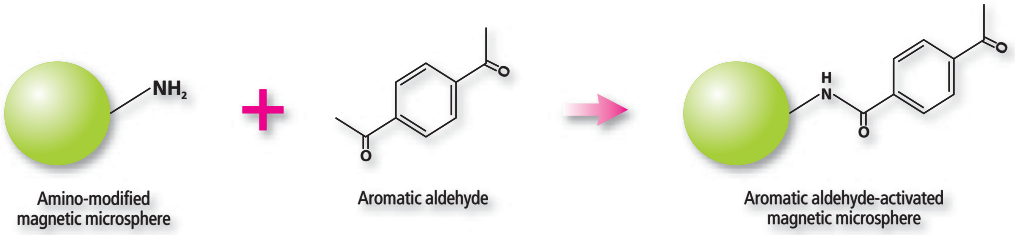
NanoLink S-4FB Magnetic microspheres are a new class of paramagnetic solid phase used to readily immobilize biomolecules. The reactive surface of the paramagnetic microspheres is modified with aromatic aldehydes that specifically react with aromatic hydrazine (HyNic) moieties attached to the surface of any biomolecule.

The microspheres can be used to immobilize:

- HyNic-activated peptides
- HyNic-modified proteins, *e.g.,* antibodies and enzyme
- HyNic-modified oligonucleotides, RNA or DNA
- HyNic-modified oligosaccarides

NanoLink™ Magnetic Separation Products

The main advantage of this aldehyde chemistry is its specificity. The aromatic aldehyde surface reacts only with HyNic-modified molecules and is insensitive to the presence of biological functionalities such as –NH2, –SH, –COOH, or –OH species. Other advantages include its long-term stability in aqueous solutions over other immobilization chemistries. NanoLink S-4FB (SFB) Magnetic Microspheres remain reactive for months without any perceptible loss of reactivity toward aromatic hydrazines. During the immobilization process they are immune to competing hydrolysis and other unwanted side-reactions. NanoLink S-4FB (SFB) Magnetic Microspheres are also ideal for automated, high throughput immobilization processes that use magnets to affect multiplex binding and separation of defined biomolecules.

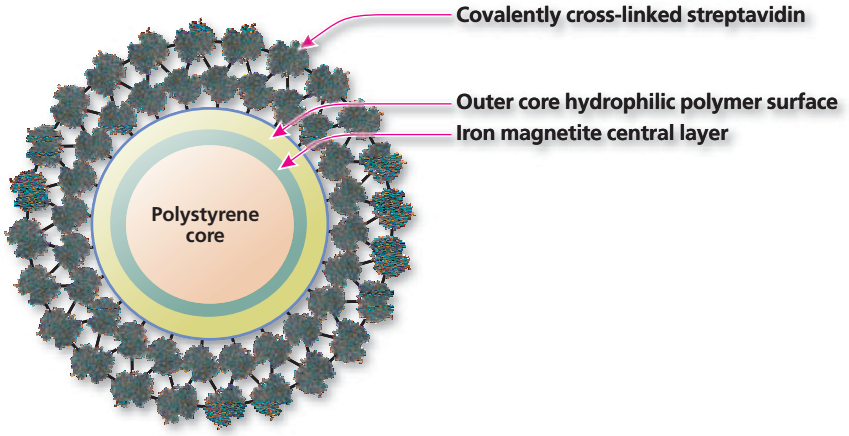


Catalog	NL-1000
Material	NanoLink™ S-4FB Magnetic Microparticles
Mfg lot #	1020-87-1
Diameter	788 nm
Magnetite content	40%
Size distribution	±5%
Aromatic-aldehyde content	26.6 nmol/mg
Particle density	1.5 g/mL
Solid content	1% solids (10 mg/mL)
Particles per mL	2.6 x 1010 (10 mg/mL)
Sodium azide	0.05%

Certificate of Analysis

NanoLink Streptavidin Magnetic Microspheres

Product Description: NanoLink Streptavidin Magnetic microspheres are uniform, polymer-encapsulated (no exposed iron), nanometer sized, super-paramagnetic particles containing covalently cross-linked streptavidin. NanoLink streptavidin magnetic microspheres are made by covalently cross-linking streptavidin to a hydrophilic surface using SoluLink's proprietary aromatic aldehyde-hydrazine conjugation chemistry. The high surface area of these paramagnetic nanoparticles when combined with SoluLink's efficient linking chemistry produces a consistent, ultra-high, biotin binding capacity per unit mass of polymer microspheres.



NanoLink™ Magnetic Separation Products

Features and Benefits

- Consistent high biotin binding capacity (>8 nmol/mg)
- Binds >2 nmol/mg biotin oligonucleotide
- Encapsulated (no exposed iron) monodispersed microspheres
- Uniform size distribution
- Fast magnetic response time (25 electromagnetic units)
- Stable in collodoidal form
- Stable in detergents
- Paramagnetic (no residual magnetism)

The particles are supplied at approximately 1% solids (10mg/ml) in nuclease-free water with 0.05% sodium azide. There are no surfactants present. Particles can be washed prior to use to remove residual azide if desired but is not necessary for most applications.

Applications: NanoLink Streptavidin Magnetic microspheres possess the highest biotin-binding capacity of any commercially available polymer-encapsulated streptavidin particle. These microspheres are particularly suited for high throughput robotic applications where high biotin loads must be removed or immobilized without the presence of an iron leachate

The microspheres can be used to immobilize:

- Biotinylated antibodies and other proteins
- Biotinylated dsDNA (gDNA, PCR products) or biotinylated aRNA
- Biotinylated oligonucleotides (> 2nmol/mg binding capacity)

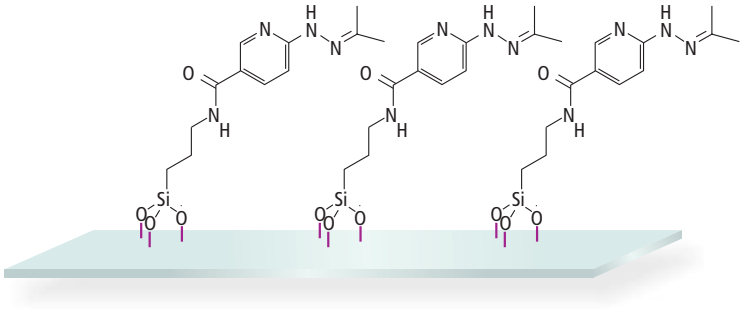
The main benefit of using these ultra-high biotin-binding microspheres is the reduction in the overall bead mass required to immobilize a biotinylated sample. This leads to both lower backgrounds of non-specific binding and lower net costs. Applications include separation of biotin-labeled biomolecules including biotin-labeled genomic DNA, RNA, PCR products, oligonucleotides, peptides, or antibodies. NanoLink Streptavidin Magnetic Microspheres are ideally suited for generating single-stranded PCR templates that can dramatically increase hybridization efficiency to complementary probes by removal of the unbiotinylated competing PCR strand.

Surface Chemistry & Immobilization

SoluLink offers a series of new HydraLink™-based products for the fail-safe immobilization of biomolecules to solid surfaces.

HyLink™ Glass Slides

SoluLink is also offering premium, HyNic-activated glass slides called HyLink Glass Slides (Catalog # HL-001). These glass slides are exclusively for the immobilization of aromatic aldehyde (4-FB) modified biomolecules. These extremely flat, 1 x 3-inch slides are ultra clean and come pre-coated with a HyNic-silane layer that efficiently immobilizes any aromatic aldehyde-modified (4-FB) biomolecule without fear of competing hydrolysis from the aqueous environment.



Pre-coated Hydrazinosilane Glass Microarray Slide

These pre-activated glass slides are an ideal solid phase substrate for the efficient microarray-based printing and immobilization of aromatic aldehyde-modified oligonucleotides and antibodies.

BeadLink™ Kit

SoluLink also offers a BeadLink Kit (Catalog # S-9005-1) containing all the necessary reagents for converting amino-modified beads to aromatic aldehyde (4-FB) beads with sulfo-S-4FB. This kit also contains the heterobifunctional linker S-HyNic used to HyNic-modify biomolecules. Simple mixing of aldehyde microspheres with a HyNic-modified biomolecule covalently immobilizes the biomolecule to the surface via hydrazone bond formation.

Surface Chemistry & Immobilization Products

Product	Description	Catalog #	Size/Quantity	Price
HyLink	Glass Slides	HL-0001	10 slides	$ 100
BeadLink	Kit	S-9005-1	Kit	$ 375
BeadLink Kit Components	S-HyNic (SANH)	S-1002	15 mg	
	Sulfo-S-4FB (SFB)	S-1004	100 mg	
	4-nitrobenzaldehyde	S-2001	100 mg	
	2-hydrazinopyradine.2 HCl	S-2002	100 mg	
	2-sulfobenzaldehyde	S-2005	100 mg	
	DMF		1.0 mL	
	10x Modification Buffer		1.5 mL	
	10x Conjugation Buffer		1.5 mL	

Finally.

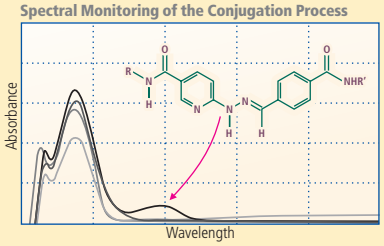
Simple and Stable Conjugation Chemistry!

The SoluLink Conjugation System

Solulink's proprietary new chemistry connects biomolecules to each other or to solid surfaces — each retaining its original capabilities, while synergistically taking on new functionality. Our conjugation chemistry has been engineered to conjugate, label, and immobilize proteins, antibodies, peptides and oligonucleotides

- **universal conjugation/ immobilization capability**
- **unmatched reproducibility**
- **spectrophotometric traceability & quantification**
- **enhanced assay perfomance**
- **easy to use with fewer steps**
- **mild chemistry preserves activity**
- **higher yields = cost effectiveness**

Spectral Monitoring of the Conjugation Process



Explore other SoluLinK innovative products like our traceable biotinylation reagent, ChromaLinK™ Biotin.This novel, new product allows simultaneous labeling and spectrophotometric measurement of biotin with one reagent.

Learn more about Solulink and our products at www.solulink.com or call us toll-free at 888.625.0670.

SoluLinK
The Conjugation Company™

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www.solulink.com

PepLink™ Linkable Peptides

"We recently ordered PepLink pre-activated catalog peptides from SoluLink and were able to get the activated peptides quicker, paid less, and were able to use them quicker than with custom peptides or catalog peptides needing subsequent conjugation work."

SoluLink
The Conjugation Company™

PepLink™ Linkable Peptides

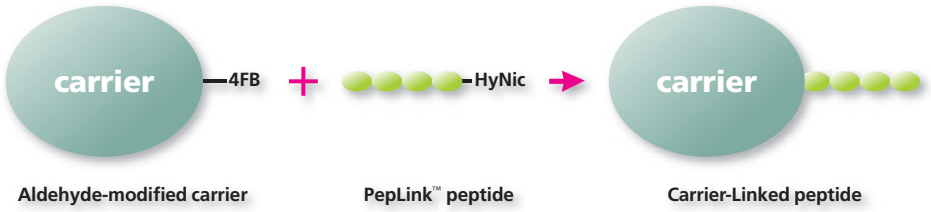
PepLink Library

- **PepLink Epitope Tags:** For Purification/Detection
- **PepLink Cell Penetrating Peptides:** Delivery
- **PepLink Therapeutic Peptides:** Disease Specific

PepLink™ Linkable Peptides — Catalog or Custom

SoluLinK Introduces a First in its Class

SoluLinK is proud to introduce a library of ready-to-link activated peptides called PepLinK linkable peptides that allow easy linking of peptides to any biomolecule. PepLink peptides come in a stable, pre-activated form so they automatically target and efficiently link to any aldehyde-modified biomolecule simply by mixing the two together.



PepLink peptides can be linked to a wide variety of biomolecules including siRNAs, antibodies, proteins, antigen carriers, *e.g.,* KLH, oligonucleotides, fluorophores, biofluorescent proteins, *e.g.*, R-phycoerythrin, and surfaces.

Features and Benefits of PepLink Linkable Peptides:

- Ready-to-ship PepLink Library or custom sequences
- PepLink peptides link only to aldehyde-modified biomolecules
- UV-traceable conjugation (linkage absorbs @ 354nm)
- PepLink's (HyNic) group can be inserted at any location within the peptide (internal, –COOH, or $–NH_2$)
- Cleavable linkers are available to release the peptide

SoluLinK has engineered PepLink linkable peptides to incorporate a proprietary, pre-activated (HyNic) linker to the peptide during solid phase synthesis. PepLink Linkable Peptides can be linked to either $–NH_2$ or –SH containing biomolecules, carriers, or surfaces that have been modified with SoluLinK's proprietary aldehyde linker (S-4FB).

Three simple steps are required to produce a conjugate

- Rapid carrier modification with an aldehyde moiety
- Incubation of the PepLink peptide with the modified carrier
- Rapid purification using spin-filter diafiltration

PepLink™ Linkable Peptides

PepLink Epitope Tags

Epitope tags, such as 6X-His can be engineered into proteins using recombinant methods. SoluLinK is now the first to provide pre-activated, linkable PepLink peptides for tagging native, non-recombinant proteins with soft-release epitope tags. PepLink epitope tags provide a non-biotin based affinity tagging method for the soft-release or detection of tagged non-recombinant proteins. SoluLinK offers four of the most popular epitope tags in two sizes for easy conjugation to proteins, oligonucleotides or other biological molecules.

PepLink Epitope Tags

Name	Sequence	Catalog #	Unit	Price
His-Tag	HyNic-(PEG_2)-$(His)_6$-$CONH_2$	SP-E001	0.5 mg	$175
			1.0 mg	$275
S-Tag	HyNic-SDMHQREFKAAAYGK-$CONH_2$	SP-E002	0.5 mg	$175
			1.0 mg	$275
c-Myc-Tag	HyNic-YGQLRNSRA-NH_2	SP-E003	0.5 mg	$175
			1.0 mg	$275
Flag-Tag	HyNic- DYKDDDDK-$CONH_2$	SP-E004	0.5 mg	$175
			1.0 mg	$275

PepLink Cell Penetrating Peptides for Intracellular Delivery

There is significant interest[1-4] in the use of cationic peptides for the intracellular delivery of proteins, nucleic acids, siRNAs, or drugs into the cytoplasm of cells. Although the exact mode of action for these cell-penetrating peptides (CPPs) has not been elucidated, their utility has been experimentally demonstrated for cytoplasmic drug delivery of therapeutic agents.

SoluLinK now introduces pre-activated PepLink CPPs that contain our proprietary HyNic linker for easy conjugation to other biomolecules. PepLink CPPs (with or without cleavable linkers) are readily conjugated to siRNAs or oligonucleotides for the study of targeted gene regulation. Often cell types respond differently to different classical cationic transfection agents so researchers routinely have to evaluate and optimize conditions for both efficient delivery and cytotoxic effects. PepLink Cell Penetrating Peptides offer an alternative to common transfection agents. SoluLinK offers two pre-activated PepLink cationic-peptides (CPPs) for easy conjugation to biological payloads and subsequent intracellular delivery and release.

PepLink Cell Penetrating Peptides

Name	Sequence	Catalog #	Unit	Price
$(Arg)_8$	HyNic-RRRRRRRR-NH_2	SP-C001	0.5 mg	$225
			1.0 mg	$375
TAT	HyNic-GRKKRRQRRRPPQ-NH_2	SP-C002	0.5 mg	$225
			1.0 mg	$375
Transportan	HyNic-GWTLNSAGYLLGKI NLKALAALAKKIL-NH_2	SP-C003	0.5 mg	$225
			1.0 mg	$375

PepLink™ Linkable Peptides

PepLink Therapeutic Peptides

Introduction

The therapeutic efficiency of any drug or peptide is dependent on various intrinsic pharmacokinetic parameters that include the rate of adsorption and/or clearance from the human body. Researchers often wish to conjugate peptides to other biomolecules as a means of improving their therapeutic properties. For example, pegylation of peptides can improve their adsorption and clearance properties by modifying and altering their stability/solubility. Pegylation can also protect them from early proteolytic digestion, and increase their biological half-life. Conjugation of peptides to other biomolecules (including other peptides) can also be used to impact their biological targeting and delivery. Bioconjugation can also be exploited to reduce or increase bioavailability. In summary, the pharmacokinetic profile of therapeutic peptides can be altered significantly by conjugating them to carriers such as biomolecules or polymers.[1]

For this reason, SoluLinK is introducing four pre-activated PepLink Therapeutic linkable peptides (HyNic-modified at the NH_2-terminus) that have potential use in therapeutic in vivo studies. These PepLink peptides target two different disease areas. One group of PepLink therapeutic peptides target Obesity (Obestatin and Ghrelin) and the other targets Alzheimer's disease [β-Amyloid (1-40) and β-Amyloid (1-42)]. PepLink peptides come pre-activated and ready to link to any other (aromatic aldehyde-modified) biomolecule by simply mixing the two together. For example, these therapeutic peptides can be conjugated to any aromatic aldehyde-modified PEG reagents, peptides, lipids, or complex polysaccarides.

PepLink Therapeutic Peptides that Target Obesity

Ghrelin

Ghrelin is a hormone produced by P/D1 cells lining the fundus of the human stomach and is known to stimulate appetite. In rodents, X/A-like cells produce ghrelin. Ghrelin levels increase before meals and decrease after meals. It is considered the counterpart of the hormone leptin, produced by adipose tissue, which induces satiation when present at higher levels. Ghrelin also stimulates the secretion of growth hormone from the anterior pituitary gland.

Receptors for ghrelin are expressed by neurons in the arcuate nucleus and the ventromedial hypothalamus. The ghrelin receptor is a G-protein coupled membrane receptor, formerly known as the GHS receptor (growth hormone secretagogue receptor). Ghrelin plays a significant role in neurotropy, particularly in the hippocampus, and is essential for cognitive adaptation to changing environments and the process of learning.

Obestatin

Obestatin is a hormone that is produced in the cells lining the stomach and small intestine of several mammals including humans; it drastically reduces appetite in mice and is expected to do the same in humans. Obestatin is a peptide hormone — a relatively small protein and is encoded by the same gene that encodes ghrelin, a peptide hormone that increases appetite. The full-length protein produced by that gene breaks into two smaller peptides, ghrelin and obestatin. The purpose of this mechanism remains unclear, however it explains earlier findings, namely that removing the ghrelin gene from mice

PepLink™ Linkable Peptides

does not significantly reduce their appetite. Obestatin could be developed into a drug against obesity, however it would have to be delivered as a nasal spray, injection, or transdermal patch as the peptide is destroyed by stomach acids.

It is known that obestatin is amidated at the C terminus, while ghrelin is acylated. Binding studies have found that amidation is needed for obestatin to bind to the G protein-coupled receptor GPR39, a member of the ghrelin receptor subfamily, and to stimulate c-AMP production. In vivo experiments found that amidated human obestatin reduced food intake in a time- and dose-dependent manner in mice and significantly reduced the body weight of rats when given alone. When combined with ghrelin, which increases body mass, obestatin inhibited weight gain. Similarly, an in vitro analysis of ghrelin and obestatin on jejunum muscle contraction confirmed ghrelin's stimulatory effect and found that obestatin reduced contractile activity when used alone and antagonized ghrelin's affect when the two are present together.

PepLink Peptides Targeting Alzheimer's Disease

β-Amyloid (1-40, 1-42) are peptides involved in Alzheimer's Disease (AD). AD is characterized by the deposition of extracellular senile plaques whose major component is amyloid β peptide (Aβ). Aβ40 to Aβ43 are 40-43 amino acid peptides that are cleaved from amyloid precursor protein (APP) during apoptosis by β-secretase, *e.g.*, BACE, and by a putative γ-secretase. Increased Aβ formation leads to the elevated extracellular concentrations of the "longer forms" of Aβ, namely Aβ 42 or Aβ43. These peptides have a greater tendency to aggregate than Aβ40 and, therefore, are considered to be pathological. The increased release of Aβ42/Aβ43 leads to the abnormal deposition of Aβ and the associated neurotoxicity in the brains of affected individuals.

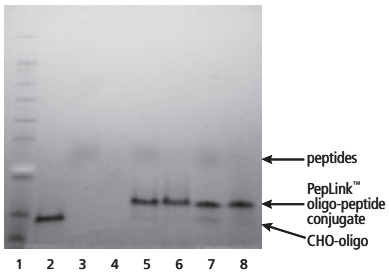
References

1. Protein, peptide and non-peptide drug PEGylation for therapeutic application. Pasut G.; Guiotto A.; Veronese F. *Expert Opinion on Therapeutic Patients,* Volume 14, Number 6, 1 June 2004, pp. 859-894(36)

PepLink Therapeutic Peptides

Name	Sequence	Catalog #	Unit	Price
Ghrelin	HyNic-FLSPEHQKAQQRKESKKP PAKLQPR	SP-0010	0.5 mg	$ 250
			1.0 mg	$ 425
Obestatin	HyNic-FNAPFDVGIKLS GAQYQQHGRAL	SP-0011	0.5 mg	$ 275
			1.0 mg	$ 450
β-Amyloid (1-40)	HyNic-KAEFRHDSGYGVHHQKLVFFA GDVGSNKGAIIGLMVGGVV-NH2	SP-0012	0.5 mg	$ 325
			1.0 mg	$ 575
β-Amyloid (1-42)	HyNic-KAEFRHDSGYGVHHQKLVFF AGDVGSNKGAIIGLMVGGVVIA-NH2	SP-0013	0.5 mg	$ 350
			1.0 mg	$ 650

PepLink™ Linkable Peptides

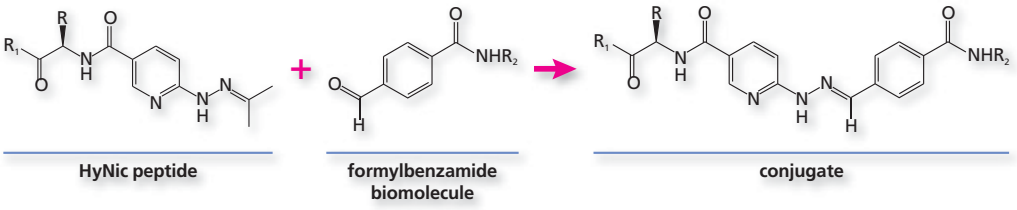


1 – Molecular weight protein marker
2 – 5′-FB-18mer oligo
3 – HyNic-PepLink™ A
4 – HyNic-PepLink™ B
5 – HyNic-PepLink™ A-Oligo conjugate
6 – HyNic-PepLink™ B-Oligo conjugate
7 – 4 (incubated at 94°C for 2 hr)
8 – 5 (incubated at 94°C for 2 hr)

FIGURE 1 • Two N-terminal PepLink peptides (A and B, lanes 3 and 4) were prepared by solid phase peptide synthesis and reacted with a 5′- benzaldehyde-modified 18-mer oligo (5′-FB) (lane 2) to yield the PepLink-oligo conjugate (lanes 5 and 6). Conjugate stability was tested at 94°C in PBS for two hours. PAGE results as visualized using UV back-shadowing. As seen in lanes 5, 6, 7, and 8, conjugation was efficient and the conjugate bond was stable to heating at 94°C.

The Chemistry that Drives HyNic-Aldehyde Conjugation

The chemistry for the formation of the HyNic-benzyaldehdye conjugates is outlined below. The hydrazone formed is stable and reduction of the hydrazone is not necessary.



Conjugating with PepLink Linkable Peptides

PepLink Peptide-Oligo Conjugation

PepLink Peptide-Protein Conjugation

PepLink peptides are readily conjugated to a protein by modifying with SoluLinK's S-4FB reagent. This linker reagent efficiently incorporates benzaldehyde moieties on lysine-containing proteins. Simple addition of a HyNic-modified PepLink peptide to the benzaldehyde-modified protein yields the desired PepLink peptide-protein conjugate. The number of PepLink peptides conjugated to the protein (molar substitution ratio) is readily quantified, *i.e.*, UV-traceable verification. A simple spectral scan at 354 nm quantifies hydrazone bond formation.

In Figure 2 below, a 15-mer HyNic-modified PepLink peptide was conjugated to benzaldehyde-modified ovalbumin.

PepLink Peptide-Protein Conjugation

PepLink peptides are readily conjugated to a protein previously modified with SoluLinK's S-4FB reagent or other reagent that incorporates 4-FB on biomolecules or surfaces. These linker reagents efficiently incorporate 4-FB moieties on lysine-containing proteins. Simple addition of a HyNic-modified PepLink peptide to the benzaldehyde-modified protein yields the desired PepLink peptide-protein conjugate. The number of PepLink peptides conjugated to the protein (molar substitution ratio) is readily quantified, *i.e.*, UV-traceable verification). A simple spectral scan at 354 nm quantifies hydrazone bond formation.

PepLink Conjugation Protocols

Complete PepLink conjugation protocols are available. Refer to SoluLinK's Conjugation Calculators and Protocols on our web site (www.solulink.com)

PepLink technology is also available through our Contract Conjugation Services. These services include SoluLinK's Custom Peptide, Oligonucleotide, and RNA synthesis.

PepLink™ Linkable Peptides

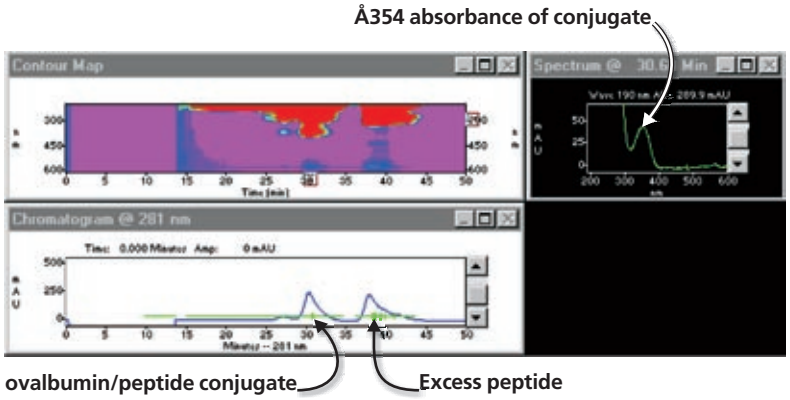


FIGURE 2 • A 15-mer HyNic-modified PepLink peptide was reacted with benzaldehyde-modified ovalbumin. The conjugate was purified by size exclusion chromatography equipped with a photodiode array detector. The UV spectral chromatogram ($Å_{354}$, top right) of the separation verifies conjugate bond formation (hydrazone bond) between the PepLink peptide. The UV chromatogram ($Å_{280}$, bottom) reveals separation of the excess PepLink peptide from the conjugate.

Related PepLink Linkable peptides products and accessories

PepLink linkable peptides are associated with a family of complimentary and related products that expand their functional performance capabilities.

Cleavable & Non-Cleavable Linkers	S-4FB (SFB) (S-1004)
	SS-S-4FB (SFB) (S-1037-025)
Peptide-linkable fluorophores & biofluorescent proteins	Fluorescein-FB (S-4006)
	Rhodamine-FB (S-4007)
	Texas Red-FB (S-4005)
	4FB-Phycoerythrin (R-PE) (S-4008)
	4FB-Allophycocyanin (APC) (S-4009)
Anti-epitope Tag Antibodies	anti-His (S-4010)
	anti-flag (S-4011)
	anti-cMyc (S-4012)
NanoLink Magnetic Particles	NanoLinK-S-4FB (SFB) (M-1001-001)
PEGylation of PepLink Peptides	PEG4-Benzaldehyde (4FB) (S-1039)
	PEG12-Benzaldehyde (4FB) (S-1040)
	PEG24-Benzaldehyde (4FB) (S-1042)
	Biotin/PEG3/Benzaldehyde(4FB)(S-1042)
PepLink Peptide Lipids	Cholesteryl/PEG/4FB (S-5003)
	Steroyl-4FB PE-4FB (S-5002)
	4FB Lipids (S-5001)

PepLink™ Linkable Peptides

References

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2 Torchilin VP., Recent approaches to intracellular delivery of drugs and DNA and organelle targeting. *Annu Rev Biomed Eng.* 2006;8:343-75 (Review)

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Quantification & Quenching Reagents

"SoluLinK's quantification and quenching reagents worked as advertised and allowed us to quantitate the amount of modification we had on our targets."

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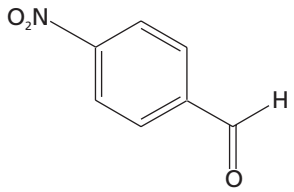
Quantification & Quenching Reagents

4-Nitrobenzaldehyde

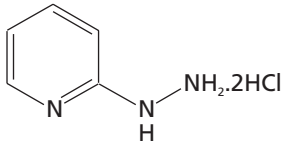


$C_7H_5NO_3$ — MW 151.1 — yellow solid — 98% purity by HPLC

Application: Used to colorimetrically quantitate the level of hydrazine and hydrazide modification on biomolecules.

Product	Description	Catalog #	Size/Quantity	Price
4-Nitrobenzaldehyde	Reagent	S2001-100	100 mg	$ 150
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/4nitro			
Product Data Sheet	www.solulink.com/productdatasheet/4nitro			
MSDS	www.solulink.com/msds/4nitro			

2-Hydrazinopyridine.dihydrochloride



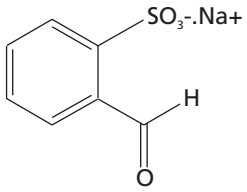
$C_5H_6N_3$.HCl — MW 182.1 — yellow solid — 98% purity by HPLC

Application: Used to colorimetrically quantitate the level of aldehyde incorporation

Product	Description	Catalog #	Size/Quantity	Price
2-Hydrazinopyridine.dihydrochloride	Reagent	S2002-100	100 mg	$ 150
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/2hydrazinopyridine			
Product Data Sheet	www.solulink.com/productdatasheet/2hydrazinopyridine			
MSDS	www.solulink.com/msds/2hydrazinopyridine			

Quantification & Quenching Reagents

2-Sulfobenzaldehyde



$C_7H_5O_4SNa$ (technical grade 70%) — MW 208.2 — white solid — 98% purity by HPLC

Application: Used to quench hydrazine and hydrazide moieties on modified biomolecules.

Product	Description	Catalog #	Size/Quantity	Price
2-Sulfobenzaldehyde	Reagent	S2005-100	100 mg	$ 150
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/2sulfo			
Product Data Sheet	www.solulink.com/productdatasheet/2sulfo			
MSDS	www.solulink.com/msds/2sulfo			

Introducing
ChromaLink Biotin 354S

Biotinylate & Quantify Labeling of Antibodies & Surfaces with Just **One** Reagent!

- **Direct biotin measurement for reproducible quantification.**
- **Faster and easier than the traditional HABA : Avidin displacement method.**
- **Very small aliquots provide reproducible results.**

Application

ChromaLink (catalog # B-1001) incorporates biotin on amine-containing biomolecules or surfaces and allows for direct spectrophotometric quantitation of total biotin incorporation on any biomolecule. By measuring the Å280 and Å354 of the modified biomolecule, the protein concentration and number of biotins incorporated/protein can be determined directly. As little as 20 µg aliquot of modified protein in 100 µL buffer in a microplate assay will yield reproducible results.

ChromaLink Biotinylation Kit
B1001-02K kit
Includes:
- ChromaLink 2 x 0.5 mg
- DMF (anhydrous) (1 mL)
- 10X modification buffer (2 mL)
- 2 x 5K MWCO diafiltration apparatus

For more information about our reagents and services, visit our website or call us at 858.625.0670.

SoluLinK Biosciences, Inc.
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The Conjugation Company™

Peptide & Miscellaneous Reagents

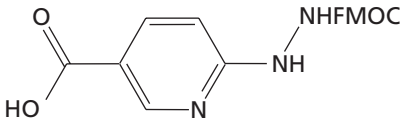
"We used SoluLink's 6-Fmoc-HNA to incoprorate their HyNic (6-hydrazinonicotinamide) linker onto the N-terminal amino groups of peptides we were synthesizing. The reagents were convenient to use and allowed us to make further modifications after final synthesis."

SoluLinK
The Conjugation Company™

Peptide & Miscellaneous Reagents

6-Fmoc-HNA

(6-Fmoc-hydrazinonicotinic acid)



$C_{21}H_{17}N_3O_4$ — MW 375.2 — yellow solid 98% by HPLC

Product Description: Used to incorporate HyNic (6-hydrazinonicotinamide) moieties during solid or solution phase peptide synthesis or used to directly incorporate Fmoc-protected HNA onto a small molecule, surface or polymer where base-labile protection is required. 6-Fmoc-HNA is a heterobifunctional linker used to convert primary amines to Fmoc-protected HyNic (6-hydrazinonicotinamide) moieties during solid phase peptide synthesis. This linker does not require activation for incorporation.

Application: Reagent for the introduction of the 6-Fmoc-HNA group during Fmoc SPPS. It can be coupled to the N-terminal amino group as the last step in the synthesis, prior to cleavage, or coupled to the side-chain of a Lys residue which was introduced using an appropriately orthogonally protected derivative such as Lys(ivDde) or Lys(Mtt).

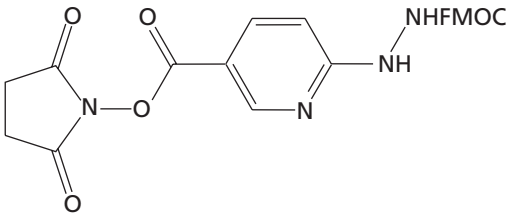
Product	Description	Catalog #	Size/Quantity	Price
6-Fmoc-HNA	Reagent	S-3004-100	100 mg	$ 195
	Reagent	S-3004-250	500 mg	$ 495
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/6fmochna			
Product Data Sheet	www.solulink.com/productdatasheet/6fmochna			
MSDS	www.solulink.com/msds/6fmochna			

This product is protected by US Patents 5,206,370, 5,420,285, 5,753,520, and 5,769,778, and EU Patent 0.384,769.

Peptide & Miscellaneous Reagents

6-Fmoc-HNA-OSu

(Succinimidyl 6-Fmoc-hydrazinonicotinate)



$C_{25}H_{20}N_4O_6$ — MW 472.2 — yellow solid — 98 % purity by HPLC

Application: 6-Fmoc-HNA-OSu is a heterobifunctional linker used to convert primary amines to Fmoc-protected HyNic (6-hydrazinonicotinamide) moieties during solid phase peptide synthesis. This linker does not require activation for incorporation. 6-Fmoc-HNA-Osu is used to directly incorporate Fmoc-protected hydrazine into a peptide, small molecule, surface, or polymer where base-labile aromatic hydrazine protection is required.

Features

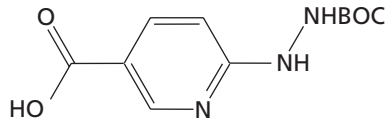
- Heterobifunctional linker
- Used during automated peptide synthesis
- Introduction of Fmoc-protected aromatic hydrazine to NH2-terminus of peptides during synthesis
- Reactive groups: NHS-ester, Fmoc-protected aromatic hydrazine
- Reactive towards: amino groups, aromatic aldehydes

Product	Description	Catalog #	Size/Quantity	Price
6-Fmoc-HNA-OSu	Reagent	S-3001-100	100 mg	$ 225
	Reagent	S-3001-500	500 mg	$ 595
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/6fmochnaosua			
Product Data Sheet	www.solulink.com/productdatasheet/6fmochnaosu			
MSDS	www.solulink.com/msds/6fmochnaosu			

Peptide & Miscellaneous Reagents

6-Boc-HNA

(6-Boc-hydrazinonicotinic acid)



$C_{11}H_5N_3O_4$ — MW 253.1 — pale yellow solid — 98% purity by HPLC

PRODUCT DESCRIPTION: Reagent for the introduction of the 2-hydrazinopyridine group during Boc. It can be coupled to the N-terminal amino group as the last step in the synthesis, prior to cleavage, or coupled to the side-chain of a Lys residue which was introduced using an appropriately orthogonally protected derivative such as Lys(ivDde) or Lys(Mtt).

APPLICATION: 6-Boc-HNA is a homofunctional linker used to incorporate Boc-protected HyNic (6-hydrazinonicotinamide) moieties during solid phase peptide synthesis. This linker requires activation for incorporation during peptide synthesis. 6-Boc-HNA is used to incorporate Boc-protected hydrazine into a peptide, small molecule, surface, or polymer where acid-labile aromatic hydrazine protection is required.

Features

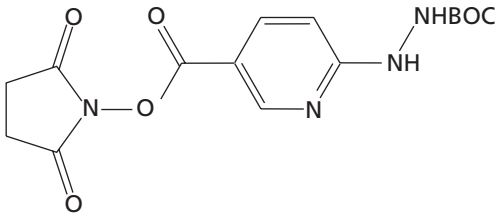
- Introduction of Boc-protected aromatic hydrazine to NH2-terminus of peptides during synthesis
- Reactive groups: COOH, Boc-protected aromatic hydrazine
- Reactive towards: amino groups, aromatic aldehydes

Product	Description	Catalog #	Size/Quantity	Price
6-Boc-HNA	Reagent	S3003-100	100 mg	$ 195
	Reagent	S3003-500	500 mg	$ 495
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/6bochna			
Product Data Sheet	www.solulink.com/productdatasheet/6bochna			
MSDS	www.solulink.com/msds/6bochna			

Peptide & Miscellaneous Reagents

6-Boc-HNA-OSu

(Succinimidyl 6-Boc-hydrazinonicotinate)



$C_{15}H_{18}N_4O_6$ — MW 350.3 — pale yellow solid — 98% purity by HPLC

Product Description: Reagent for the introduction of the 2-hydrazinopyridine group during Boc. It can be coupled to the N-terminal amino group as the last step in the synthesis, prior to cleavage, or coupled to the side-chain of a Lys residue which was introduced using an appropriately orthogonally protected derivative such as Lys(ivDde) or Lys(Mtt).

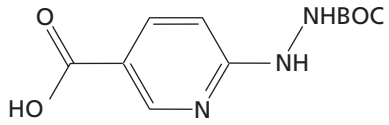
Application: 6-Boc-HNA is a homofunctional linker used to incorporate Boc-protected HyNic (6-hydrazinonicotinamide) moieties during solid phase peptide synthesis. This linker requires activation for incorporation during peptide synthesis. 6-Boc-HNA is used to incorporate Boc-protected hydrazine into a peptide, small molecule, surface, or polymer where acid-labile aromatic hydrazine protection is required.

Product	Description	Catalog #	Size/Quantity	Price
6-Boc-HNA-OSu	Reagent	S-3002-100	100 mg	$ 225
	Reagent	S-3002-250	250 mg	$ 495
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/6bochnaosu			
Product Data Sheet	www.solulink.com/productdatasheet/6bochnaosu			
MSDS	www.solulink.com/msds/6bochnaosu			

Peptide & Miscellaneous Reagents

Boc BTSC

(4-Boc-thiosemicarbazido benzoate)



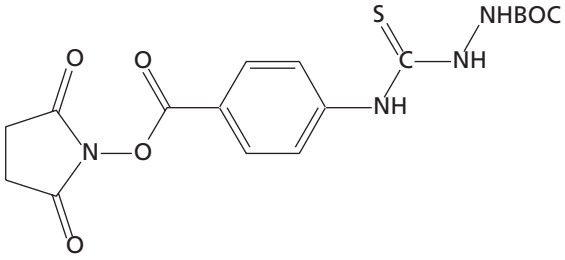
$C_{13}H_{17}N_3O_4S$ — MW 331.36 — white powder — 98% by HPLC

Application: Used to incorporate Boc-protected 4-thiosemicarbazidobenzamide moieties (4-BTSC) during solid or solution phase peptide synthesis.

Product	Description	Catalog #	Size/Quantity	Price
Boc BTSC	Reagent	S-1018-100	100 mg	$ 295
	Reagent	S-1018-250	250 mg	$ 495
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/bocbtsc			
Product Data Sheet	www.solulink.com/productdatasheet/bocbtsc			
MSDS	www.solulink.com/msds/bocbtsc			

Boc BTSC OSu

(Succinimidyl 4-Boc-thiosemicarbazido benzoate)



$C_{17}H_{20}N_4O_6S$ — MW 408.34 — white powder — 98% purity by HPLC

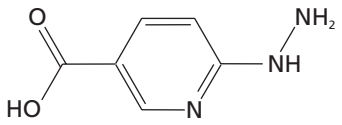
Application: Used to incorporate Boc-protected 4-thiosemicarbazidobenzamide moieties (4-BTSC) during solid or solution phase peptide synthesis without activation or used to directly incorporate Boc-protected 4-thiosemicarbazidobenzamide onto a small molecule or polymer where acid-labile protection is required.

Product	Description	Catalog #	Size/Quantity	Price
Boc BTSC OSu	Reagent	S-1016-100	100 mg	$ 295
	Reagent	S-1016-250	250 mg	$ 595
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/bocbtscosu			
Product Data Sheet	www.solulink.com/productdatasheet/bocbtscosu			
MSDS	www.solulink.com/msds/bocbtscosu			

Peptide & Miscellaneous Reagents

6-HNA

(6-hydrazinonicotinic acid)



$C_6H_7N_3O_2$ — MW 153.1 — yellow solid — 98% purity by HPLC

Product Description: Used to incorporate HyNic moieties during solid or solution phase peptide synthesis.

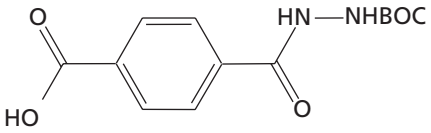
Application: Water-compatible reagent for the modification of amino-functionalized surfaces or amine-containing biomolecules, such as proteins, peptides, or oligonucleotides, prior to bioconjugation with HydraLink™ S-4FB-modified biomolecules.

Product	Description	Catalog #	Size/Quantity	Price
6-HNA	Reagent	S2003-100	100 mg	$ 295
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/6hna			
Product Data Sheet	www.solulink.com/productdatasheet/6hna			
MSDS	www.solulink.com/msds/6hna			

This product is protected by US Patents 5,206,370, 5,420,285, 5,753,520, and 5,769,778, and EU Patent 0.384,769.

Boc-HTA

(4-Boc-hydrazido-terephthalic acid)



$C_{13}H_{16}N_2O_5$ — MW 280.3 — white solid — 98% purity by HPLC

Product Description: Used to incorporate aromatic hydrazide moieties during solid or solution phase peptide synthesis.

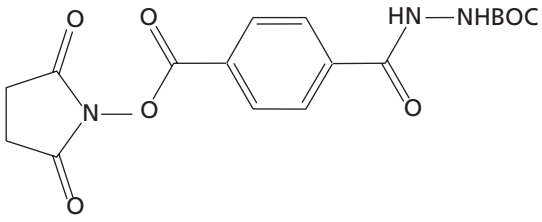
Application: Used to incorporate aromatic hydrazide moieties during solid or solution phase peptide synthesis.

Product	Description	Catalog #	Size/Quantity	Price
Boc-HTA	Reagent	S-1014-100	100 mg	$ 250
	Reagent	S-1014-500	250 mg	$ 450
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/bochta			
Product Data Sheet	www.solulink.com/productdatasheet/bochta			
MSDS	www.solulink.com/msds/bochta			

Peptide & Miscellaneous Reagents

Boc-HTA-OSu

(Succinimidyl 4-Boc-hydrazidoterephthalate)



$C_{17}H_{19}N_3O_7$ — MW 377.4 — white solid — 98%

PRODUCT DESCRIPTION: Used to incorporate hydrazine moieties during solid or solution phase peptide synthesis.

APPLICATION: Used to incorporate aromatic hydrazide moieties during solid or solution phase peptide synthesis.

Product	Description	Catalog #	Size/Quantity	Price
Boc-HTA	Reagent	S-1015-100	100 mg	$ 250
	Reagent	S-1015-500	250 mg	$ 495
Other Materials Required	None			
Related Products	None			
Protocol	www.solulink.com/protocols/bochtaosu			
Product Data Sheet	www.solulink.com/productdatasheet/bochtaosu			
MSDS	www.solulink.com/msds/bochtaosu			

Custom Conjugation Services

Let Solulink do your conjugations for you. Solulink's expert staff of chemists and biochemists can quickly conjugate any of your biomolecules to one another or to solid surfaces like chips, slides, plates or beads for you. For your convenience, we can also provide purification, analytical information and certificate of analysis documentation. SoluLink's Custom Conjugation Services are very cost effective and allow you to move forward more quickly with your projects.

Types of Conjugates

- Protein/protein conjugation – antibody to AP or HRP or PE
- iPCR antibody-oligo conjugations
- Antibody – to any fluorophore or enzyme
- cDNA/fluorophore conjugation
- Oligonucleotide/bead conjugation
- Oligonucleotide/fluorophore conjugation
- Solid surface attachments – solid surface attachment: agarose, microbeads, magnetic beads, chips, nanobars, array, *etc*.

Streptavidin, Biotinylation, Protein A, Affinity Ligand Custom Conjugations

Biotin is the conjugate of choice if the antibody is going to be used in a variety of applications. Biotin is a small molecule that has little effect on an antibody's immunoreactivity. Its small size makes biotin an excellent tag for your antibody in most techniques. Biotin binds tightly to avidin or streptavidin, which are available enzyme conjugated, making your biotinylated antibody useful in most immunological procedures. The disadvantage of a biotinylated antibody over an enzyme or fluorescent conjugated antibody is that there is an additional step involved in your assay.

Enzyme Linkages with AP, HRP, or Other Enzymes

Enzyme conjugates give a highly selective and sensitive immuno-reagent. By directly labeling your primary antibody you eliminate steps and washes. Alkaline Phosphatase (AP) or Horseradish Peroxidase (HRP) is a stable enzyme suitable for ELISA, Western Blot and immunocytochemistry. AP and HRP have low endogenous activity and a high turnover rate, producing its colored end product in a short time period.

Fluorescent Markers

Fluorescent probes are used in multiple probe assays, immunofluorescence microscopy and flow cytometry. A minimum quantity of 200 µg of antibody is required for all fluorescent tagging.

Custom Conjugation Services

Examples of Service Pricing & Options

Prices vary, inquire for your specific project or for volume discounts.

Protein – Protein Conjugation Service < 3 mg		**$ 1500**
Options	Protein G Purification (> 0.5 mg antibody)	
	Ordering of Proteins or Antibodies	
	Activity Assay	
	PAGE Gel	
	New Column Packing	

Antibody (Random Protein) – Oligo Conjugation Service		**$ 1500**
Options	Protein G Purification (>0.5 mg antibody)	
	Ordering of the Oligos or Antibodies	
	Activity Assay	
	PAGE Gel	
	New Column Packing	

AP – Oligo Conjugation Service		**$ 1500**
Options	Ordering of the Oligos	
	Activity Assay	
	PAGE Gel	
	New Column Packing	

HRP – Oligo Conjugation Service		**$ 650**
Options	Ordering of the Oligos	
	Activity Assay	
	PAGE Gel	
	New Column Packing	

Peptide – Oligo Conjugation Service		**$ 1500**
Options:	Ordering of the Peptides or Oligos	
	PAGE Gel	
	New Column Packing	

Service prices, detailed specifications, and further information will be provided upon request. A project manager will be assigned and report to you regularly on an agreed schedule.

To initiate a Custom Contract Service Project, contact our Technical Services Department at info@solulink.com or 888.625.0670.

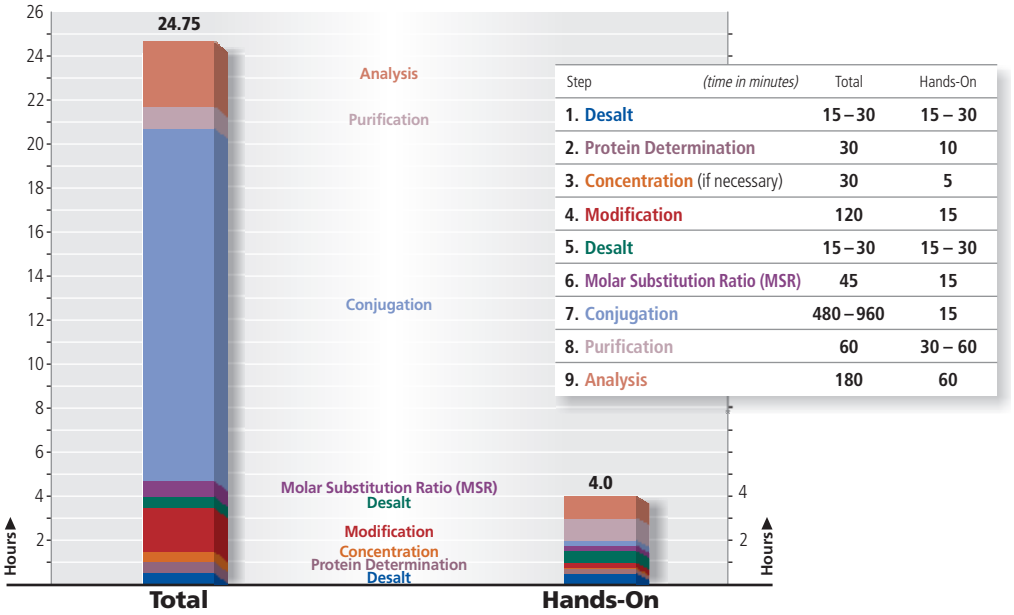
Example Protocol

SoluLinK's bioconjugation process is a straightforward nine step process used with many different types of biomolecules including antibodies, carbohydrates, peptides, lipids, oligonucleotides, RNA, DNA, fluorophores, and other small molecules. This section presents an example of the protocol using a protein-protein conjugation.

SoluLinK
The Conjugation Company™

Example Protocol

SoluLinK's HydraLink™ technology can be used to conjugate many different types of biomolecules including antibodies, carbohydrates, peptides, lipids, oligonucleotides, RNA, DNA, fluorophores or other small molecules. The HydraLink bioconjugation process is a straightforward nine step process. As an example, we show in this section a protein-protein conjugation. Figure 1 details the steps with an overview of time requirement. Methods and protocols for each of these steps is included in this section.



Step	(time in minutes)	Total	Hands-On
1. Desalt		15 – 30	15 – 30
2. Protein Determination		30	10
3. Concentration (if necessary)		30	5
4. Modification		120	15
5. Desalt		15 – 30	15 – 30
6. Molar Substitution Ratio (MSR)		45	15
7. Conjugation		480 – 960	15
8. Purification		60	30 – 60
9. Analysis		180	60

FIGURE 1 • Overview of the time and the standardized steps used to produce a protein-protein conjugate.

Current SoluLinK bioconjugation kits currently provide reagents for five of the nine steps: 1, 4, 5, 6, and 7. We will soon provide reagents for all nine steps — so check back for updates.

The end-user provides reagents for four of the nine steps: (2) protein determinations, (3) concentration, (8) conjugate purification, and (9) conjugate analysis. In the following pages, SoluLinK includes methods and protocols that we routinely employ for all nine steps. By sharing our knowledge and experience, we intend to remove any confusion with HydraLink bioconjugation process and thus aid researchers to reach their ultimate goal of bioconjugate-driven discoveries.

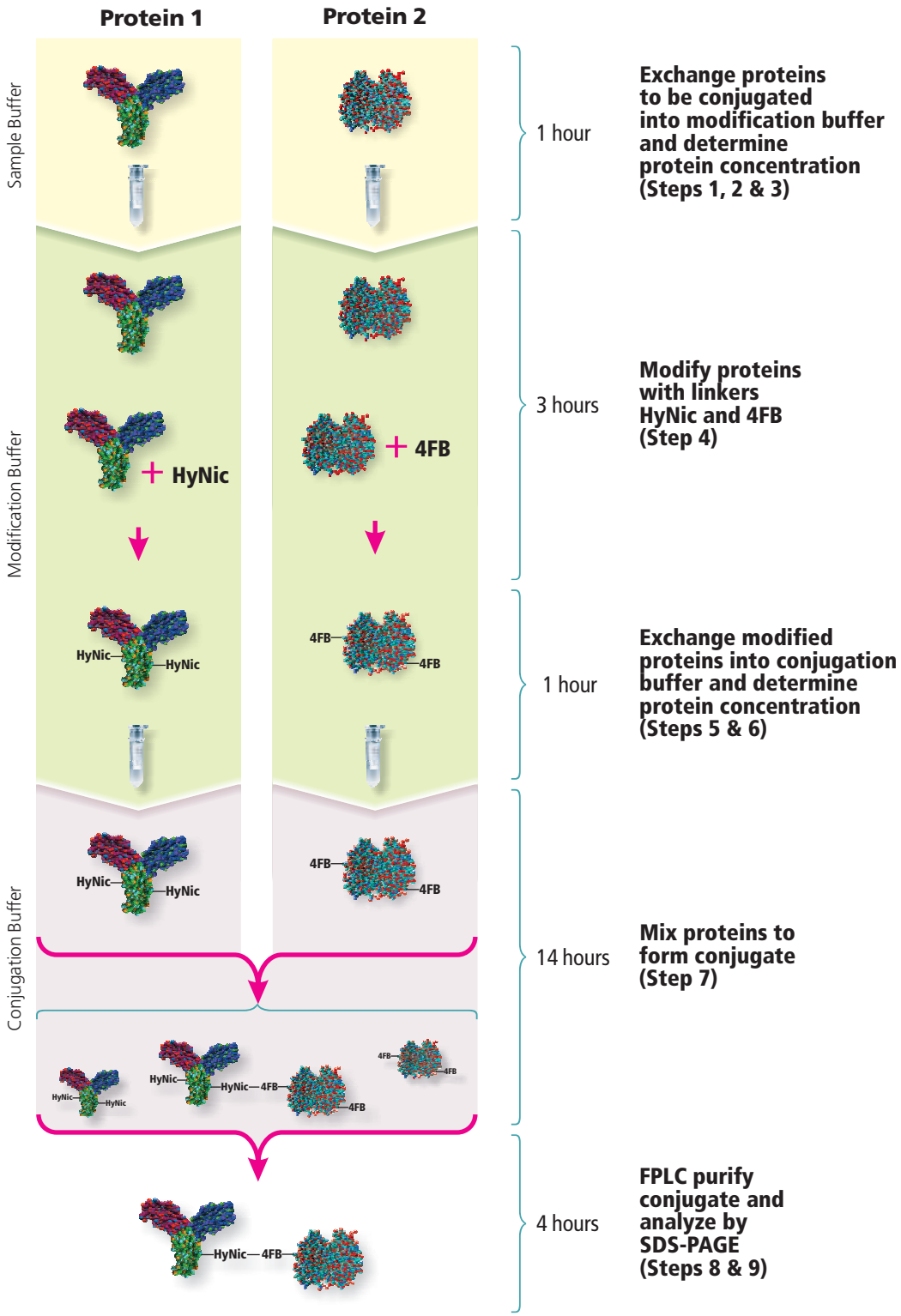


Figure 2 • Overview of the conjugation process.

Example Protocol

1. Buffer Exchange/Desalt

Once it is determined that both proteins are at the appropriate initial concentration and volume, the first step is to desalt the two proteins being conjugated. All proteins require desalting before and after modification with linkers. Desalting proteins prior to modification insures that any potential (and often present) amine contaminants will not interfere with the modification process. This step should never be omitted. Desalting is also carried out after protein modification with the linkers. Post-modification desalting is used to remove excess un-reacted equivalents of linker and to exchange the protein from a slightly basic modification buffer (pH 7.2) into a slightly acidic conjugation buffer (pH 6.0).

2. Protein Concentration Determination

The second step in the conjugation process is determination of a protein's concentration. The concentration of all proteins must be determined after desalting and prior to modification. Protein concentrations are required for determining the number of linker equivalents added to a modification reaction.

3. Protein Concentration (Optional)

HydraLink kits perform best when operating within defined protein concentrations of 2–5 mg/mL and volumes of 130–500 µL. When necessary, commercial diafiltration spin filters are used to rapidly concentrate diluted protein samples.

4. Modification

In this crucial step, previously desalted and quantified proteins are chemically modified with SoluLinK's two heterobifunctional linkers S-HyNic and S-4FB. The HydraLink system modifies a protein's lysine amino groups through the linker's NHS ester functionality. The S-4FB linker introduces aldehydes into a protein and the S-HyNic linker introduces hydrazines. In practical terms, it does not matter which protein is modified with hydrazines (S-HyNic) and which is modified with aldehydes (S-4FB) as long as the two functional groups are located on different proteins.

5. Exchange into Conjugation Buffer/Desalt

After modification, proteins are once again desalted to remove excess linker and to exchange the proteins into a suitable conjugation buffer.

Example Protocol

6. Molar Substitution Ratio (MSR) (Optional)

After exchange, aliquots of each modified protein are analyzed for protein concentration (BCA) and the molar substitution ratio (MSR). Two colorimetric reagents are used to determine and quantify incorporated aldehydes and hydrazines followed by simple absorbance readings to determine the MSR.

7. Conjugation

Conjugation involves mixing the two modified proteins together at known equivalents and allowing them to react for 8–16 hours. A crude reaction mixture containing the desired conjugate is the end result of this step. This crude mixture normally contains several species of conjugate along with any excess modified proteins that were not linked during the conjugation process. Crude conjugation reactions are usually analyzed on SDS-PAGE or non-denaturing gradient protein gels prior to purification and analysis.

8. Purification

Purification is usually carried out on chromatographic gel filtration media (Superdex 200) or ion exchange (Q-Sepharose). Purified column fractions are monitored using a diode-array detector.

9. Analysis

Analysis includes spectral analysis of purified fractions using a diode array detector that can readily verify hydrazone bond formation. Aliquots of collected fractions are analyzed using SDS-PAGE or Bis-Tris protein gradient gels. Spectral analysis unequivocally identifies conjugate peak identity and often their quantitative purity. Gel analysis is used to confirm and benchmark conjugate size and yield. Both reaction and purification conditions often allow 1:1 heterodimers, 1:2 heterotrimers, large heteropolymers, or mixtures thereof to be isolated in pure form on demand.

Example Protocol

General Guidelines

Modification of Proteins with S-HyNic (SANH) or S-4FB (SFB)

As stated above, the number of functional groups incorporated per protein molecule is commonly referred to as the molar substitution ratio, or MSR. The final MSR obtained is a function of several complex variables that include protein concentration, number of available amino-groups on the protein, number of S-HyNic (or S-4FB) equivalents added, reaction pH, isoelectric point of the protein, local protein hydrophilicity/hydrophobicity index, and, to a much lesser degree, the type of heterobifunctional NHS-ester employed.

In general, as the protein concentration and number of equivalents are increased the molar substitution ratio increases. Caution is recommended however, since over-modification changes the isoelectric point of the protein and can result in catastrophic precipitation or loss of biological activity. Since not all proteins are created equal, optimization of S-HyNic (SANH) and S-4FB (SFB) modification conditions may be required in certain instances or if more or less of a given heteropolymeric molecular weight is desired, *e.g.*, 1:1,1:2, and 1:3 conjugates. *A priori*, no hard and fast rules exist for unknown proteins, nonetheless as an aid in determining the number of equivalents of S-HyNic (SANH) (or S-4FB (SFB)) needed to achieve a given molar substitution ratio (MSR), SoluLinK has assembled data based on simple matrix experiment. This experiment relied on the use of a UV-traceable NHS-reagent to quantify incorporation ratios (Table 1). This table is intended to serve as a general guide for modifying proteins however each case could have its own variables. The table summarizes the molar substitution ratios (MSRs) obtained using bovine IgG and a UV-traceable NHS ester.

Specifically, the experiment examined the effects of pH (7.2 and 8.0), protein concentration (1.0, 2.5. and 5.0 mg/mL) and the number of equivalents (5, 10 and 15 equivalents) on substitution ratios (MSR). Reactions conditions used 1 x Modification Buffer [100 mM phosphate, 150 mM NaCl (pH 7.2) or 100 mM HEPES, 150 mM NaCl (pH 8.0)] in 50 µL for 2 hours at room temperature. All protein concentrations were determined using the BCA protein assay and a spectrophotometer to quantify the degree of modification (MSR) @ 350 nm. The data for this particular protein (IgG) illustrates that initial protein concentration appears to have the most significant impact on final MSR.

IgG mg/mL	Equiv. Added	MSR (pH 7.2)	MSR (pH 8.0)
1.0	5	2.38	2.07
	10	4.73	4.05
	15	6.20	6.14
2.5	5	3.08	2.91
	10	6.58	5.85
	15	8.26	7.59
5.0	5	3.74	3.34
	10	6.8	6.04
	15	9.76	8.51

Table 1 • General guide for achieving a "targeted MSR" using heterobifunctional.

Example Protocol

Steps 1 & 5: Desalt Protocol

NOTE: SoluLinK recommends that all proteins and other amine-containing biomolecules always be desalted prior to and after modification by exchanging into 1 x Modification Buffer (100 mM sodium phosphate, 150 mM NaCl, pH 7.4).

Spin Column Preparation

1. Remove column's bottom closure and loosen the top cap (do not remove cap).
2. Place column in the 1.5 mL microfuge collection tube.
3. Centrifuge at 1,500 x g for 1 minute to remove storage solution.
4. Place a mark on the side of the column where the compacted resin is slanted upward. Place column in the microfuge with the mark facing outward in all subsequent centrifugation steps.
5. Add 300 µL of molecular grade water on top of the resin bed. Centrifuge at 500 x g for 1 minute to remove liquid.
6. Repeat step 4 two additional times, discarding buffer from the collection tube.
7. Column is now equilibrated with molecular grade water and ready for sample loading.

Sample Loading

1. Place the column in a new 1.5 mL collection tube, remove cap and slowly apply 125 µL of sample volume to the center of the compact resin bed.

   Note: *for sample volumes less than 125 µL , add additional ultra-pure water (stacker) to the top of the resin bed after the sample has fully absorbed to ensure maximal protein recovery. Avoid contact with the sides of the column when loading.*

2. Centrifuge at 1,500 x g for 2 minutes to collect desalted sample.
3. Discard desalting column after use.
4. Protein sample is now desalted and ready for addition of 10x Modification or 10x Conjugation buffer.

Troubleshooting

Problem	Possible Cause	Solution
Sample or buffer does not flow through resin	Centrifugation problem	*Ensure that centrifuge is in proper working condition*
		Ensure bottom closure is removed
Sample contamination	Improper sample loading	*Load sample directly to the center of the resin bed. Carefully touch pipette tip to resin to expel all sample.*
		Avoid contact with sides of column
	Centrifugation problem	*Do not exceed recommended speed or centrifugation time*
Low yield	No stacker used	*Apply a 15 µL water or buffer stacker after the sample has entered the gel bed*

Example Protocol

Step 2: Protein Determination Protocol

Protein determinations are a routine part of virtually every bioconjugation project. Although other biomolecules (DNA, oligonucleotides, peptides, or carbohydrates) are often conjugated using HydraLink™ kits, the overwhelming type of reaction centers on linking these and other biomolecules to proteins. For this reason, BCA protein assay reagents and protocols are provided.

Although several assays are available for determining protein concentration, SoluLinK recommends the BCA protein assay (reduction of copper in bicinchoninic acid solution) to measure concentrations. This reagent (a trademark of Pierce Chemical, Rockford, Illinois) is rapid, simple, and highly reproducible. We routinely employ a 96-well plate format for this assay that greatly expedites absorbance measurements. Many common plate readers come equipped with standard curve analysis software that help determine protein concentrations using this reagent.

BCA Protein Assay

Preparation of BCA Working Reagent

Reagents		
BCA Reagent A	5 mL	96-well polystyrene plate
BCA Reagent B	100 µL	40°C water bath
BSA standard:	2 mg/mL in 1 x PBS (1 mL)	1 x PBS (10 mL)

1. Prepare a working solution of BCA reagent by adding 5 mL BCA reagent A to a 15 mL conical tube.
2. Add 100 µL BCA reagent B and mix. A clear green solution will form.

   Note: *Prepare this working reagent fresh daily.*

96-Well BCA Protein Assay Protocol

1. Prepare a 2-fold standard curve dilution series using a 2 mg/mL BSA standard (1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL, 0 mg/mL) in a clean 96-well polystyrene plate as follows:

   Well #1: *Add 50 µL 1 x PBS and 50 µL 2 mg/mL BSA standard to a well (1 mg/mL)*

   Well #2: *Add 50 µL 1 x PBS and 50 µL from the 1st well to the 2nd well (0.5 mg/mL)*

   Well #3: *Add 50 µL 1 x PBS and 50 µL from the 2nd well to the 3rd well (0.25 mg/mL)*

   Well #4: *Add 50 µL 1 x PBS and 50 µL from the 3rd well to the 4th well (0.125 mg/mL)*

   Well #5: *Add 50 µL 1 x PBS and 50 µL from the 4th well to the 5th well (0.0625 mg/mL)*

   Well #6: *Add 50 µL 1 x PBS to the 6th well (Buffer blank).*
2. After BSA dilutions are complete, transfer 20 µL of each of the above solutions to a corresponding well containing 150 µL BCA working reagent.
3. Prepare 50 µL of the protein sample to be assayed by diluting into 1 x PBS buffer. The approximate concentration of the protein should be within the range of the BSA standards, *e.g.*,1–0.0625 mg/mL. Record the dilution factor.

Example Protocol

4. Samples are measured by transferring 20 µL aliquots of the protein into two wells containing 150 µL BCA working reagent (duplicates)

   Example 1: *Transfer 5 µL of a ~2.5 mg/mL protein sample to 45 µL 1 x PBS (1:10 dilution factor), mix, make duplicate wells by transferring 20 µL of this dilution into wells containing 150 µL BCA working reagent.*

   Example 2: *Transfer 25 µL of a 0.2 mg/mL protein solution to 25 µL 1 x PBS, mix, and make duplicate wells by transferring 20 µL of this dilution to two wells containing 150 µL BCA working reagent.*

   Note: *protein samples may need to be diluted (more or less) depending on their initial concentration such that the final dilution falls inside the range of the BSA standards.*

5. Seal the samples in the 96-well plate using the clear adhesive film and incubate the plate at 40°C in a water bath for 20 minutes (Figure 3).

6. Remove the plate from the bath, dry the bottom of the plate to remove excess water and proceed to measurement on a plate reader, *e.g.*, Molecular Devices, at 562 nm. A typical 96-well BCA protein assay result is shown in Figure 4.

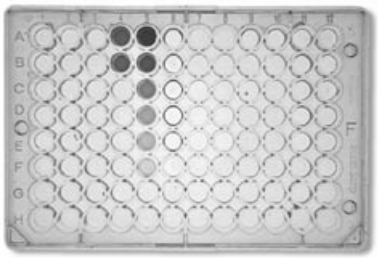


Figure 3 • 96-well BCA protein assay plate. The plate contains a dilution series of the BSA standard curve (A5 through F5) and two sample wells (A4 and A5).

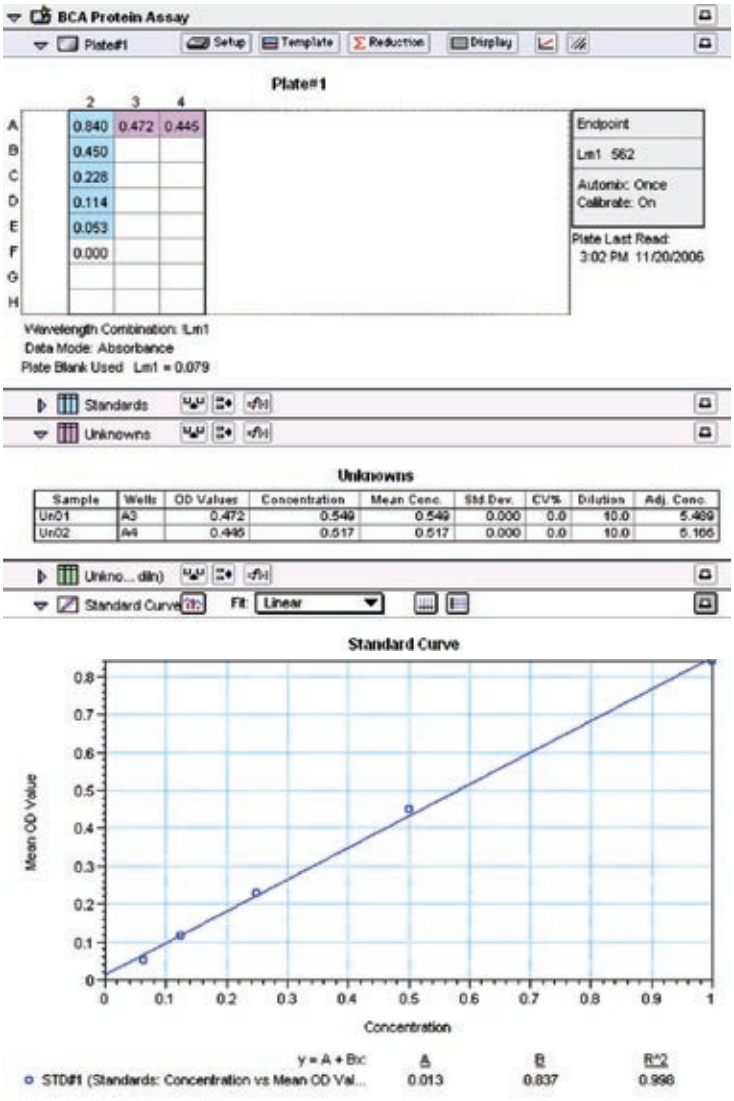


Figure 4 • BCA assay printout from Molecular Devices plate reader.

Example Protocol

FIGURE 5 • Spin filter used to concentrate dilute protein samples prior to conjugation.

Step 3: Concentration of Proteins Protocol

1. Dilute protein solutions, *e.g.*, 0.2–0.9 mg/mL, to be conjugated must first be concentrated using the diafiltration spin filter device shown in Figure 5. This spin filter is composed of a PES (polyethersulfone) membrane. This membrane has very low protein binding properties with a molecular weight cut-off (MWCO) of 5 kD. This device is made to contain and process a volume of 500 µL or less.

   Note: *For best conjugation results, the final concentrated protein should be in the 2–5 mg/mL range and a volume of 0.125 –0.5 mL*

2. To concentrate your protein sample, transfer 500 µL (or less) of dilute protein solution into the concentrator body. If the protein to be concentrated is in a volume of less than 500 µL do not adjust the volume in the concentrator.

   Note: *When transferring solutions to the concentrator body, make sure not to contact or puncture the membrane in the process.*

3. Centrifuge for 8–10 minutes @ 15,000 x g. It may be necessary to centrifuge for longer periods of time if the volume after centrifugation does not bring the protein concentration into the desired 2–5 mg/mL range.

   Note: *when using a fixed angle rotor make sure to orient the concentrator inside the microfuge so that the volume markers on the concentrator body face toward the center of the centrifuge rotor).*

4. Discard the flow through from the filtrate tube. The concentrated protein sample within the concentrator body is often in a volume of ~30–50 µL.

5. If the new reduced volume has made the protein concentration too high, add a sufficient volume of 1 x Modification buffer to bring the protein concentration to the desired range (2–5 mg/mL) range.

6. The protein sample is now desalted. Carefully transfer the desalted protein sample from the concentrator body to a clean, 1.5 mL microfuge tube. Determine the final protein concentration.

   Note: *The instructions below can be used to prepare protein-protein and other conjugates using the HydraLink S-HyNic (SANH) Bioconjugation Kit. SoluLinK recommends that this protocol be used in concert with SoluLinK's "Modification Calculators" located on our website as a Microsoft Excel spreadsheet.(www.solulink.com/technology.htm)*

Example Protocol

Step 4: Modification Protocol

Modification Protocol (S-4FB (SFB) or S-HyNic (SANH))

1. Using an analytical balance, weigh between 2.0–4.0 mg S-4FB or S-HyNic (at required equivalents) as determined by SoluLinK's modification calculators (www.solulink.com/support.htm) into a clean, 1.5 mL polypropylene tube.
2. Dissolve the S-4FB (or S-HyNic) by adding 100–200 µL DMF (see calculator).
3. Select the desired mass (and volume) of previously desalted protein in 1 x Modification Buffer to a new 1.5 mL microfuge tube as determined with the aid of the appropriate SoluLinK modification calculator (www.solulink.com/support.htm).

   Note: *PBS (10 mM phosphate, 150 mM sodium chloride, pH 7.2) is not recommended for protein modifications due to poor buffering of this low concentration phosphate buffer.*

   Note: *It is preferable to have the final protein concentration at 1–5 mg/mL range during the modification reaction.*
4. Add the appropriate volume of linker/DMF solution (desired equivalents) with the aid of SoluLinK's modification calculator and vortex briefly to mix.

   Note: *Insure the percentage of DMF in the final modification reaction does not exceed 5% of the total reaction volume.*
5. Incubate the reaction at room temperature for 2 hours.
6. Proceed to desalt the modification reaction (refer to Step 1: Desalt).

Example Protocol

Step 5: Desalt

See Step 1.

Step 6: Molar Substitution Ratio (MSR) Protocol

Hydrazine MSR

1. Prepare a 0.5 mM solution of p-nitrobenzaldehdye solution in MES buffer (pNB reagent) solution by dissolving 5 mg p-nitrobenzaldehyde in 100 µL DMF. Add a 75 µL aliquot of this solution to a 50 mL volume of 100 mM MES Buffer, pH 5.0. Label 0.5 mM p-NB solution, protect from light and keep refrigerated. This solution remains stable for 30 days at 4°C.

   Note: *Other buffers may need to be used if protein precipitation occurs due to the isoelectric point of the protein. For example, 100 mM citrate buffer (pH 6) can be used to replace 100 mM MES Buffer (5.0).*

2. Transfer 10 µL of the hydrazine-modified (desalted) protein solution (2–5 mg/mL) to a 1.5 mL microfuge tube containing 490 µL p-NB reagent the and incubate at 37°C for 0.5 hour at 37°C or 2 hours at room temperature.

   Note: *In rare instances that depend on the protein concentration of the desalted protein being measured and the final degree of S-HyNic (SANH) modification, it may require a volume greater or lesser than 10 µL of protein to get an accurate $Å_{390}$ reading on the spectrophotometer*

3. Using a 1 mL quartz cuvette, blank the spectrophotometer using 500 µL of the 0.5 mM p-NB solution in MES (without protein).

4. Remove the p-NB/protein solution from 37°C incubation and transfer the 500 µL contents to the clean quartz cuvette.

5. Determine the $Å_{390}$ of the p-NB/protein solution against the blank.

6. Calculate the hydrazine/protein MSR using the S-HyNic-Protein Modification Calculator (Step 4)found at (www.solulink.com/support.htm) or calculate the MSR by determining the hydrazine concentration using the known molar extinction coefficient of the hydrazone chromophore (24,000) and dividing by the known protein concentration.

Example Protocol

Benzaldehyde MSR

1. Prepare a 0.5 mM solution of 2-hydrazinopyridine-2HCl solution in MES Buffer (2-HP reagent) solution by dissolving 5 mg p-nitrobenzaldehyde in 100 µL DMF. Add a 91 µL aliquot of this solution to a 50 mL volume of 100 mM MES Buffer, pH 5.0. Label 0.5 mM 2-HP solution, protect from light and keep refrigerated. This solution remains stable for 30 days at 4°C.

   Note: *Other buffers may need to be used if protein precipitation occurs due to the isoelectric point of the protein. For example, 100 mM citrate (pH 6) can be used to replace100 mM MES Buffer (5.0).*

2. Transfer 10 µL of the aldehyde-modified (desalted) protein solution (2–5 mg/mL) to a 1.5 mL microfuge tube containing 490 µL p-NB reagent the and incubate at 37°C for 0.5 hour at 37°C or 2 hours at room temperature.

Note: In rare instances that depend on the protein concentration of the desalted protein being measured and the final degree of S-4FB modification, it may require a volume greater or lesser than 10 µL of protein to get an accurate $Å_{350}$ reading on the spectrophotometer.

3. Using a 1 mL quartz cuvette, blank the spectrophotometer using 500 µL of the 0.5 mM p-NB solution in MES (without protein).
4. After the reaction is complete remove the p-NB/protein solution from 37°C incubation and transfer the 500 µL contents to a clean quartz cuvette.
5. Determine the $Å_{350}$ of the 2-HP/protein solution against the blank.
6. Calculate the hydrazine/protein MSR using the S-4FB-Protein Modification Calculator (06) found at (www.solulink.com/support.htm) or calculate the MSR by determining the hydrazine concentration using the known molar extinction coefficient of the hydrazone chromophore (18,000) and dividing by the known protein concentration.

Example Protocol

Step 7: Conjugation Protocol

Successful conjugation of modified biomolecules (hydrazine/aldehyde) is dependent on several variables including biomolecule concentration, degree of modification (MSR), buffer pH, reaction time, and the ionic strength of the solution. Optimal conjugation kinetics (hydrazone formation) occurs at pH 4.7, however the conjugation reaction can proceed from pH 4.7 to pH 8.0, albeit more slowly at higher pH. HyNic and benzaldehyde functional groups have extended stability in aqueous solution therefore the concentration of the starting proteins in the conjugation reaction can be as low as 0.1 mg/mL, although we generally recommend 1 mg/mL. Conjugation reactions are normally carried out at room temperature but higher or lower temperatures can also be used.

The efficiency and extent of cross-links between two biomolecules, *e.g.*, proteins, is a function of initial protein concentration, MSR (number of reactive groups/protein), pH, reaction time, and the number of protein equivalents driving conjugate formation. For example, if one or both proteins have five functional groups/protein, a conjugate of very high molecular weight is often produced.

The final molecular weight of a conjugate can be manipulated to some extent. Conjugation reactions are often optimized in small-scale pilot reactions using a series of different linker equivalents (S-HyNic or S-4FB) and conjugating under different conditions, *e.g.*, different protein-protein equivalents. Conjugate formation is initiated by simply mixing the desired equivalents of a hydrazine-modified protein at ≥1 mg/mL with an aldehyde-modified protein at ≥1 mg/mL to yield the final conjugate. Crude reactions are then ready for analysis.

1. Conjugating two proteins requires the following:
    a. a benzaldehyde-modified protein (desalted into 1 x Conjugation Buffer) at a known protein concentration (BCA @ 1–5 mg/mL), and a previously quantified MSR.
    b. a hydrazine-modified protein (desalted into 1 x Conjugation Buffer) at a known protein concentration (BCA @ 1–5 mg/mL) and a previously quantified MSR.

    Note: *Various conjugation buffers can be used to conjugate proteins. Refer to the appendix in this catalog.*
2. Once requirements in Step 1 (a & b) have been met, the protein-protein conjugation calculators located at (www.solulink.com/technology.htm) are used to determine the appropriate mass (and volume) of each modified protein required to start the conjugation reaction.
3. Mix the appropriate mass and volume of each modified protein and incubate at room temperature for 8–16 hours.
4. If desired, the kinetics of the conjugation reaction can be determined from aliquots of the reaction mixture using conventional SDS-PAGE or Bis-Tris protein gradient (4–12%) gels. The molecular weight of conjugates can be estimated using appropriate protein molecular weight markers and visualized using any numberof staining methods (Coomassie blue, SimplyBlue™) (2–5 µg), silver (Plus-One™ Silver Stain) (~10 ng), or fluorescent stains (Sypro™ Orange) (~10 ng).
5. Generally, after an overnight incubation at room temperature the conjugate is ready for purification (FPLC) and PAGE gel analysis.

Example Protocol

Step 8: Purification

After conjugation, crude conjugation reactions are ready for purification. At SoluLinK, various FPLC or HPLC chromatography workstations have been used to purify conjugates. Typically, conjugates are purified by gel filtration or ion exchange chromatography on an HPLC-equipped with a scanning diode array detector. The detector on this workstation is capable of continuous, real-time spectral monitoring of the elution profiles from 200 to 600 nm. This detector simplifies the purification process by simultaneously "tracking" eluted conjugate peaks at several wavelengths, *e.g.*, 260 nm DNA signature, 280 nm protein signature, and 350 nm conjugate signature). Multi-wavelength elution profiles allow eluted fractions to be rapidly evaluated and pooled for further protein gel analysis.

Chromatographic Purification of Protein-Protein Conjugates

Protein-protein and protein-peptide conjugates are routinely isolated and purified using a low-pressure gel filtration column (Superdex 200, (10 cm x 40 cm). Standard run conditions are 1.25 mL/min for 0.5 hour in 1x PBS buffer (pH 7.2). Injection volumes range from 0.1 to 1 mL at a protein concentration of 1–2.5 mg/mL (for this size column). Conjugate peaks are monitored using the scanning diode array detector and specific conjugate peaks are collected and pooled according to their UV-350 signatures (hydrazone bond) as shown in Figure 6.

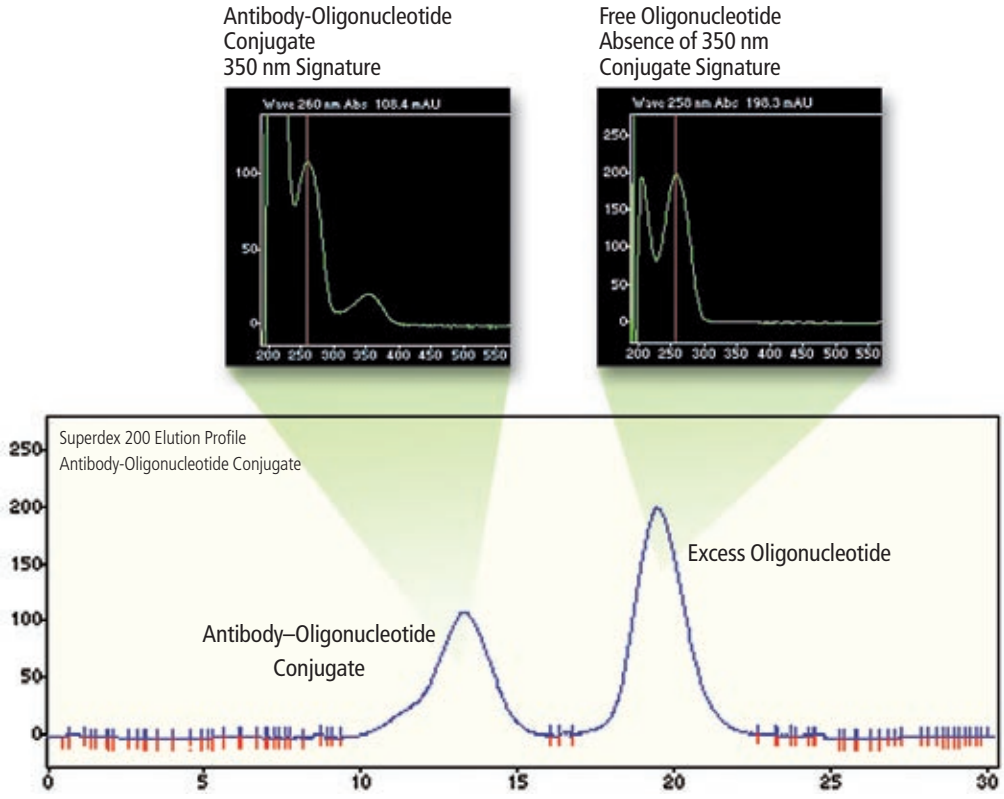


Figure 6 •UV-spectral elution profiles of a crude antibody-protein conjugate reaction (Superdex 200) gel filtration column.

Example Protocol

Chromatographic Purification of Protein-Oligonucleotide Conjugates

Protein-oligonucleotide conjugates are routinely isolated and purified using low-pressure ion exchange column chromatography (Sepharose Q, 10 cm x 40 cm). Standard run conditions use a buffered gradient (100%–300 mM NaCl, 20 mM Tris-HCl pH 8.0) to (100% 700 mM NaCl, 20 mMTris-HCl pH 8.0) at a flow rate of 1.25 mL/min for 1.5 hour. Injection volumes on this size column are 0.1–1 mL at protein concentrations of 1–2.5 mg/mL. A typical antibody-oligonucleotide elution profile is illustrated in Figure 7. Profiles were generated using the scanning diode array detector. DNA-protein conjugate peaks were monitored and detected by both their 260 and 350 nm UV signatures.

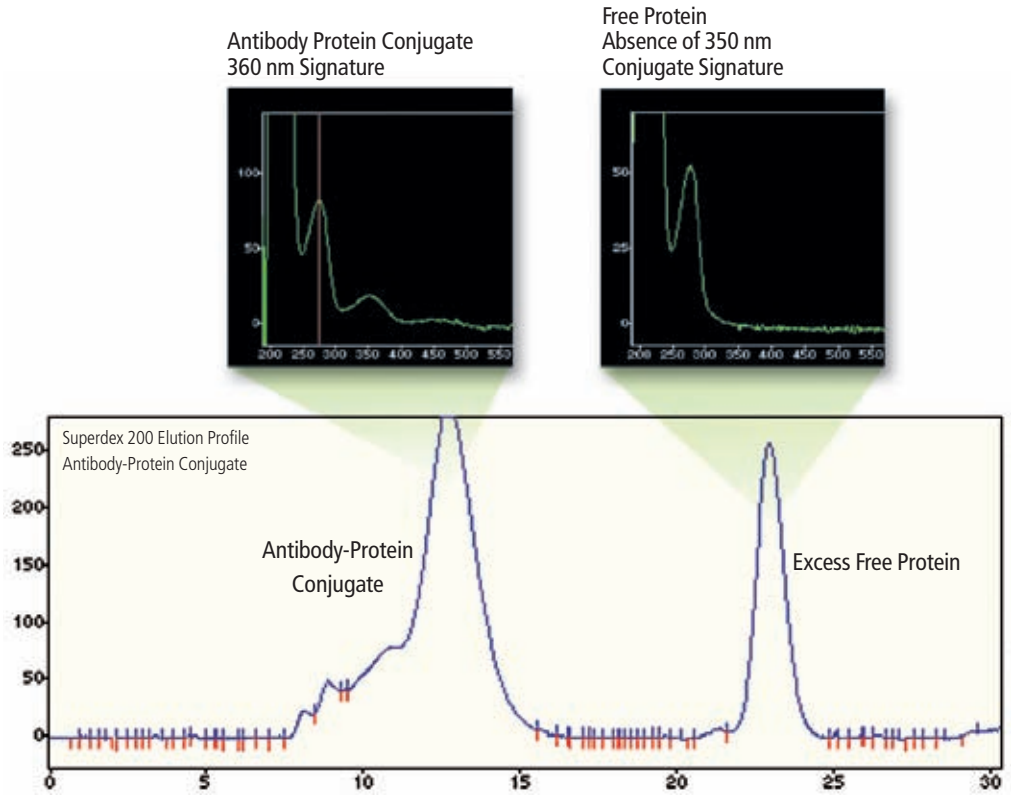


FIGURE 7 • UV-spectral elution profiles of a crude antibody-oligonucleotide conjugate reaction (Superdex 200) gel filtration column.

Example Protocol

Step 9: Analysis of Conjugates

Crude and purified conjugation reactions are typically analyzed for size and yield using a commercially pre-cast gel system. NuPAGE® (Invitrogen, Figure 8) offers a convenient and rapid polyacrylamide system for high performance gel electrophoresis and conjugate analysis. NuPAGE Bis-Tris pre-cast gels are for small to mid-size molecular weight proteins and NuPAGE Tris-Acetate gels are useful for larger proteins. Unique buffer formulations offer a low operating pH during electrophoresis that has significant advantages over other gel systems. For example, both gels systems provide a much lower pH enviro nment than traditional SDS-PAGE. A lower pH offers sharper protein band resolution, longer shelf-life, and higher accuracy.

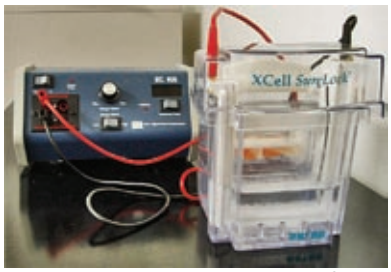


Figure 8 • XCell SureLock™ Mini-Cell rig for running pre-cast NuPAGE® gels.

For many years, the Laemmli system was the standard method used to perform SDS-PAGE. However, the Laemmli system is not available in a convenient pre-cast gel system due to gradual hydrolysis of the polyacrylamide at pH 8.7 (band distortion and/or loss of resolution). Another rawback is that the pH of the separating region of that gel system is ~9.5 (during electrophoresis). At that pH, proteins are potentially subject to chemical modifications such as deamination and alkylation. Finally, the redox state of Laemmli gels are not well controlled which makes disulfides more prone to reoxidation, giving rise to diminished band sharpness of cysteine-containing proteins.

In summary, we recommend the NuPAGE Bis-Tris and the new NuPAGE Tris-Acetate gels discontinuous SDS-PAGE systems. NuPAGE operates in a similar fashion to traditional Tris-Glycine gels, but are cast at a lower pH (pH 6.4 in Bis-Tris gels and pH 7.0 in Tris-Acetate gels). NuPAGE Bis-Tris gels do not contain SDS (instead SDS is provided in the running buffer) but are nonetheless formulated for denaturing gel electrophoresis applications only. NuPAGE Bis-Tris gels are available in three acrylamide concentrations: 10%, 4–12%, and 12%.

Conjugates and other protein samples are loaded using 4x LDS sample buffer (pH 8.4) and heated at 70°C for 10 minutes prior to loading. Run buffer consists of 1x MOPS-SDS or 1x Tris-Acetate SDS.

Typical loading and run conditions (as recommended by the manufacturer) are summarized in Invitrogen's quick reference guide.

The amount of protein loaded per lane varies depending on whether the gel will be stained with silver stain (GE HealthCare Plus One DNA Silver Staining Kit) or with Coomassie stain. Typically, we load 5–10 µg of protein if we are detecting the gels with Coomassie stain and 1 µg if we are using the silver stain. Suitably sized, pre-stained protein molecular weight markers are used (Bio-Rad), but other markers can be used.

Sample Calculator

Online calulators at www.solulink.com/support.htm

SoluLinK provides interactive calculators online at www.solulink.com/support.htm. These calculators make the modifications with our linkers and conjugates simpler by estimating the amounts of the materials needed to perform conjugations. The following example illustrates the steps in one such calculator.

Refer to the instructions on the opposite page to use the calculator.

Step	Protein Information	Input/Result	Explanation
1	Protein to be modified	BSA	Insert protein name
2	Reference #	5550102	Insert protein source/lot#
3	Modification type	Hydrazine	
4	M.W. Protein	50,000	Insert protein M.W.
5	Protein conc. (mg/mL)	2.30	Insert protein concentration (mg/mL)
	Protein Information		
6	M.W. protein (from above)	50,000	M.W. protein (as inserted above)
7	mg/mL (from above)	2.30	Protein conc. (as inserted above)
8	mg required	3.1	Insert mg of protein to be conjugated
9	Volume (µL)	1347.826	Volume (µL) protein to be used
10	mMol	6.20E-05	mMol protein reacted
11	mM	4.60E-02	mMol protein concentration
	S-HyNic (SANH) Information		
12	Lot#	S-1002	Insert S-HyNic (SANH) lot #
13	Molecular weight of S-HyNic (SANH)	290.2	M.W. S-HyNic (SANH)
14	Weight of S-HyNic (SANH) (mg)	6	Insert # mg S-HyNic (SANH) weighed
15	mMol	2.07E-02	mMol S-HyNic (SANH) weighed
16	Volume of DMF (µL)	600	Insert volume (µL) DMF added to S-HyNic (SANH)
17	Equivalents	25	Insert # equiv to be added to protein
18	mMol S-HyNic (SANH) required	1.55E-03	mMol S-HyNic (SANH) to be added to protein
19	Volume S-HyNic (SANH)/DMF required (µL)	44.98	Volume (µL) S-HyNic (SANH)/DMF added to protein solution
	Purification/Desalting		
20	Modified protein	BSA	Protein name
21	Volume of recovered Protein (mL)	1.4	Insert volume of recovered protein
22	Concentration (mg/mL)	1.9	Insert protein concentration (mg/mL)
23	Total weight recovered (mg)	2.66	Total mg recovered
24	Total mMol revccovered	5.32E-05	Total mMol recovered
25	Concentration of protein (mM)	3.80E-02	Concentration (mM) recovered protein

Sample Calculator

The following instructions detail the use of the calculator on the opposite page.

Step	**User input steps are highlighted; remaining steps are results and require no user input.**
1	Input the name of the protein to be modified with S-HyNic (SANH) reagent, e.g., BSA (Bovine serum albumin)
2	Input the commercial source or lot number of the protein to be modified.
3	Refers to the type of functional group (name) to be incorporated into the protein, e.g., hydrazine.
4	Input the known molecular weight of the protein (Daltons) to be modified with S-HyNic (SANH)
5	Input the concentration1 of the (desalted) protein, e.g., BSA, about to be modified with S-HyNic (SANH) (mg/mL).
6	Calculates the molecular weight of the protein to be modified with S-HyNic (SANH) from step 4.
7	Calculates the concentration (mg/mL) of the protein about to be modified, e.g., BSA, from step 5
8	Input the number of milligrams of protein (BSA) one wishes to conjugate
9	Calculates the volume in microliters of the protien (BSA) of step 8
10	Calculates the number of mMol of BSA to be modified
11	Calulates the concentration of the protein to be S-HyNic (SANH)-modified, e.g., BSA, in millimoles
12	Input the lot number of S-HyNic (SANH) to be used in the reaction.
13	Calculates the molecular weight of S-HyNic (SANH) reagent in Daltons
14	Input the mass of S-HyNic (SANH) weighed on the analytical balance
15	Calculates the millimoles of S-HyNic (SANH) from weight from step 14
16	Input the volume in µL of anhydrous DMF used to dissolve S-HyNic (SANH) (solid) prior to it use in the reaction
17	Input the number of mole equivalents (excess) S-HyNic (SANH) to be added relative to the protein to be modified
18	Calculates the millimoles of S-HyNic (SANH) corresponding to the number of equivalents from step 17.
19	Calculates the volume in µL of S-HyNic (SANH)/DMF reagent to be added to the protein labeling reaction required to give the millimoles shown in step 18.
20	Automatically shows the name of the S-HyNic (SANH)-modified protein from previous input
21	Input the final volume of S-HyNic (SANH)-modified protein recovered after desalting the protein/S-HyNic (SANH) reaction2.
22	Input the final desalted, S-HyNic (SANH)-modified protein concentration recovered2.
23	Calculates the total mass of S-HyNic (SANH)-modified protein recovered after desalting
24	Calculates the total number of total millimoles of S-HyNic (SANH)-modified, desalted protein recovered
25	Calculates the final S-HyNic (SANH)-modified, desalted protein concentration in millimoles

1 This value is usually determined using BCA or Bradford protein assay or by its known molar extinction coefficient.
2 This value is usually determined by BCA or Bradford protein assay.

Citations & Indices

SoluLinK
The Conjugation Company™

Citations to SoluLinK's Technology

The hydrazine linker SHNH, commonly known as HyNic, was developed for the linking of Technetium-99 to proteins for in vivo imaging[1,2]. This linker has been used to label both proteins and peptides and has been evaluated in human clinical trials. This technology is widely used and the original papers have been cited hundreds of times This novel metal binding chemistry links Technetium directly to hydrazine via a nitrogen-technetium multiple bond — not through a classical chelate bond (see references[3]).

Following on this work, SoluLinK has further expanded the utility of this linker by developing novel biomolecule linking chemistry that is the foundation of SoluLinK's technology vide supra. This technology has already been utilized in a number of applications such as immobilization of oligonucleotides to silica surfaces and beads[4,5,10], gold particles[6] and conjugation of oligonucleotides to antibodies[7], and enzymes[9].

The extensive list of citations and multi-variate uses confirms the utility of this bioconjugate chemistry.

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Master Product Index *by Category*

Master Product Index *by Category*

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Hexa-His	SP E001			
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c-myc	SP E003			
Flag	SP E004			
Glucagon 1-29	SP T001			
GST-Aromatic Aldehyde	SP E005			
Fluorophores				
Texas Red-FB	S 4005			
Fluorescein-FB	S 4006			
Rhodamine G-FB	S 4007			
BioFluorescent proteins				
R-PE (FB)	S 4008			
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Anti His	S 4010			
Anti Flag	S 4011			
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2-Sulfobenzaldehyde	S 2005			
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6-Boc-HNA (6-Boc-hydrazinonicotinic acid)	S 3003			
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Boc BTSC OSu	S 1016			
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Boc-A-AMC	S 7003			

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Notes

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How to Order

Our Customer Service Department is available 7:00 AM – 6:00 PM (Pacific Time), Monday through Friday to take and process your orders. Orders can be placed via email, telephone, fax, mail, or online at **www.solulink.com/product_ordering.htm**.

You can either use our Product Order Form to place your order or you can send in your institution's Purchase Order form. Please be sure that the following information is included when ordering:

1. Purchase order number
2. Name of institution or company
3. Customer account number (if available)
4. Shipping address
5. Billing address
6. Catalog product number and description
7. Quantity and size required
8. Name and phone number of purchasing agent
9. Name and phone number of the end user
10. Your purchase order number (if applicable)
11. Credit card number and expiration date (if applicable)

Online Orders

Please note that for ordering on-line you will be asked to provide either a Purchase Order number or a credit card number for the order to be accepted and processed.

100% Satisfaction Guarantee

Exceptional quality and batch-to-batch consistency are of paramount importance for all of us at SoluLinK. Consequently, SoluLinK has established strict quality control guidelines for each format of our products and each batch must pass these stringent biochemical and biological/immunological testing requirements.

However, if any product does not meet these specifications in your hands, please contact us; your concerns will be addressed quickly, and after investigation, the product will either be immediately replaced or credited for the original purchase price.

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The Conjugation Company™

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