Bioconjugation Resource Guide

Methodology to Linking Technology

VECTOR LABORATORIES
part of Maravai LifeSciences

Conjugate with confidence

This guide offers an overview of bioconjugation with a focus on SoluLINK® bioconjugation technology. It is intended to provide insights for researchers seeking trusted, established methodologies to capture or conjugate biomolecule(s) of interest. The products featured in this guide have provided scientists with reliable, reproducible and quantifiable results for many years.

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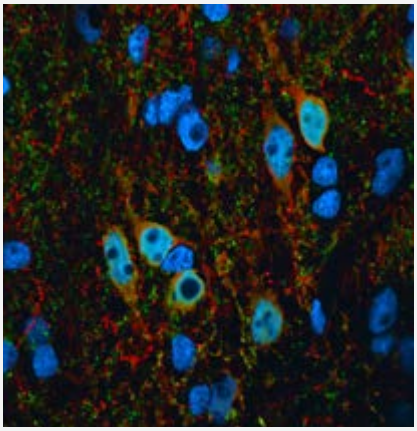
Bioconjugation Resource Guide

Vector Laboratories was founded on a growing portfolio of purified lectins and lectin conjugates that helped to pioneer lectin histochemistry. These products remain a key component of our business today. In the early 1980s, we leveraged our expertise in histochemistry to revolutionize the field of IHC with the commercialization of antibody-based avidinbiotin reagents and the introduction of the VECTASTAIN® ABC system. This system enabled routine immunohistochemistry with any standard brightfield microscope. Following the success of the ABC kits, Vector Laboratories continued to introduce novel and innovative products to support cell and tissue antigen visualization. These include the ImmPRESS® micropolymer reagents, M.O.M.® (Mouse on Mouse) detection systems, unique ImmPACT® enzyme substrates, VECTASHIELD® Antifade Mounting Media and TrueVIEW® Autofluorescence Quenching Kits for immunofluorescence applications. In early 2020, we expanded our bioconjugation portfolio with the addition of SoluLINK® products and services that include a range of conjugation kits, conjugation linkers, magnetic beads & agarose, and biotin & digoxigenin labeling reagents.



ChromaLINK Biotin and Digoxigenin-modified antibodies in a multiplexed immunofluorescence staining experiment using the same host species primary antibodies from the same host species (mouse).

Bioconjugation Introduction

Bioconjugation is the chemical linking of two molecules to form a single hybrid, where at least one of the molecules in the partnership is a biomolecule such as an antibody, protein or oligonucleotide. The resulting product retains the activity of each component yet also gains a novel function that is not possible with either molecule alone. Well known examples of bioconjugates include antibodies bound to fluorophores or enzymes; proteins attached to magnetic or agarose beads; and antibodies conjugated to oligonucleotides. These reagents are widely used to support a broad range of applications.

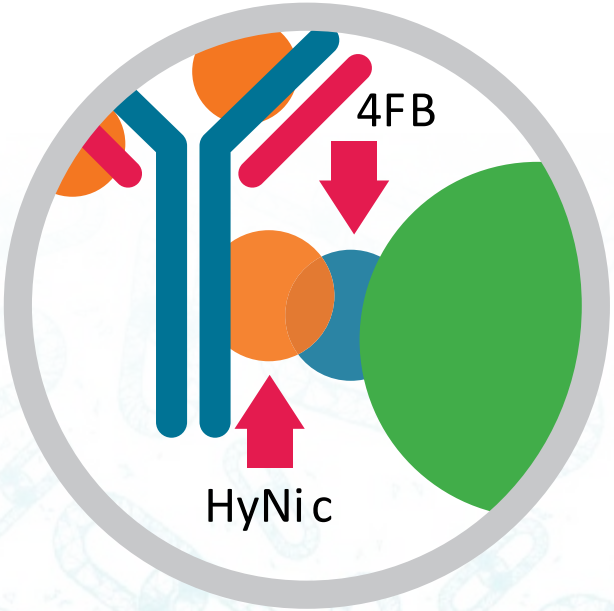
How is bioconjugation performed?

Bioconjugation typically involves adding distinct but complementary functional groups to each of the two biomolecules to enable them to bind to one another. This is achieved through a process known as modification, whereby linkers are attached to amines or thiol groups present on the biomolecules before the biomolecules are mixed together. Although performing bioconjugation has historically required an in-depth knowledge of conjugation chemistry, it is now possible to conjugate any class of biomolecule quickly and easily in-house using SoluLINK bioconjugation technology from Vector Laboratories.

What factors are key to bioconjugation success?

Biomolecules are complex materials produced by living organisms, and they exist and function only in aqueous environments. For this reason, linker attachment must occur via a mild, controllable reaction in aqueous solution (with no need for agents such as oxidants, reductants or metals) to maintain biological performance. It is also critical that no undesirable covalent side reactions occur during modification or conjugation, and that bonds be formed only between complementary linkers, not through endogenous functional groups.

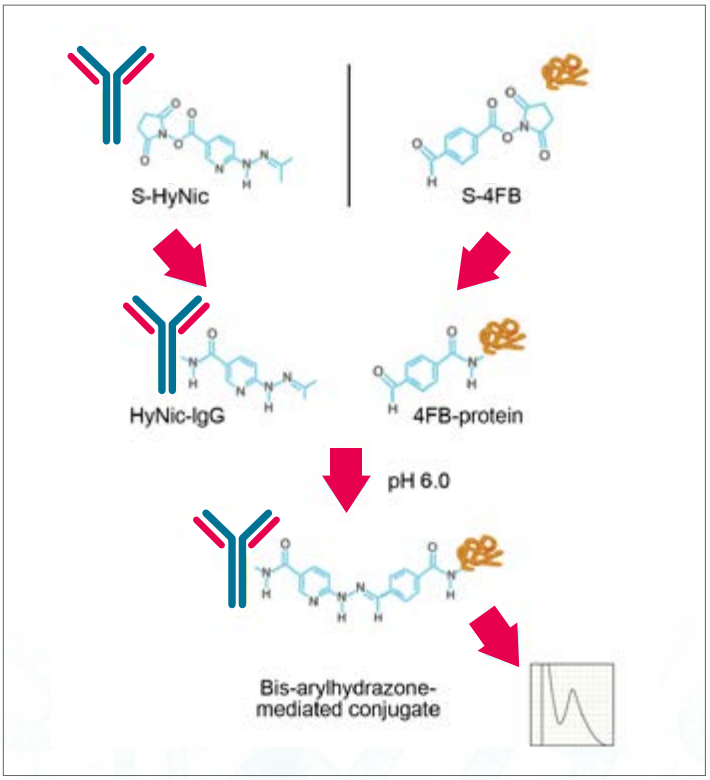
Another important consideration is that the conjugation reaction should happen directly upon mixing the two modified biomolecules together and should demonstrate fast, stoichiometrically efficient reaction kinetics. Additionally, linker incorporation and conjugate formation should be easily quantifiable through simple and non-destructive methods like spectrophotometry, while both the linker-modified biomolecules and the resulting conjugate should be stable under a broad pH range and at elevated temperatures.



Easy-to-use linker-based conjugation technology.

How does SoluLINK bioconjugation technology work?

This technology provides mild, efficient and reproducible bioconjugation of all classes of biomolecule, including antibodies, proteins, peptides, oligonucleotides, carbohydrates, drugs and surfaces. It accomplishes this by converting the amines of one molecule to aromatic hydrazine (HyNic) groups and those on the other molecule to aromatic aldehyde (4FB) groups, enabling the formation of a stable bis-arylhydrazone conjugate bond when the two molecules are combined. The bioconjugation protocols follow a simple, user-friendly protocol with minimal hands-on time.



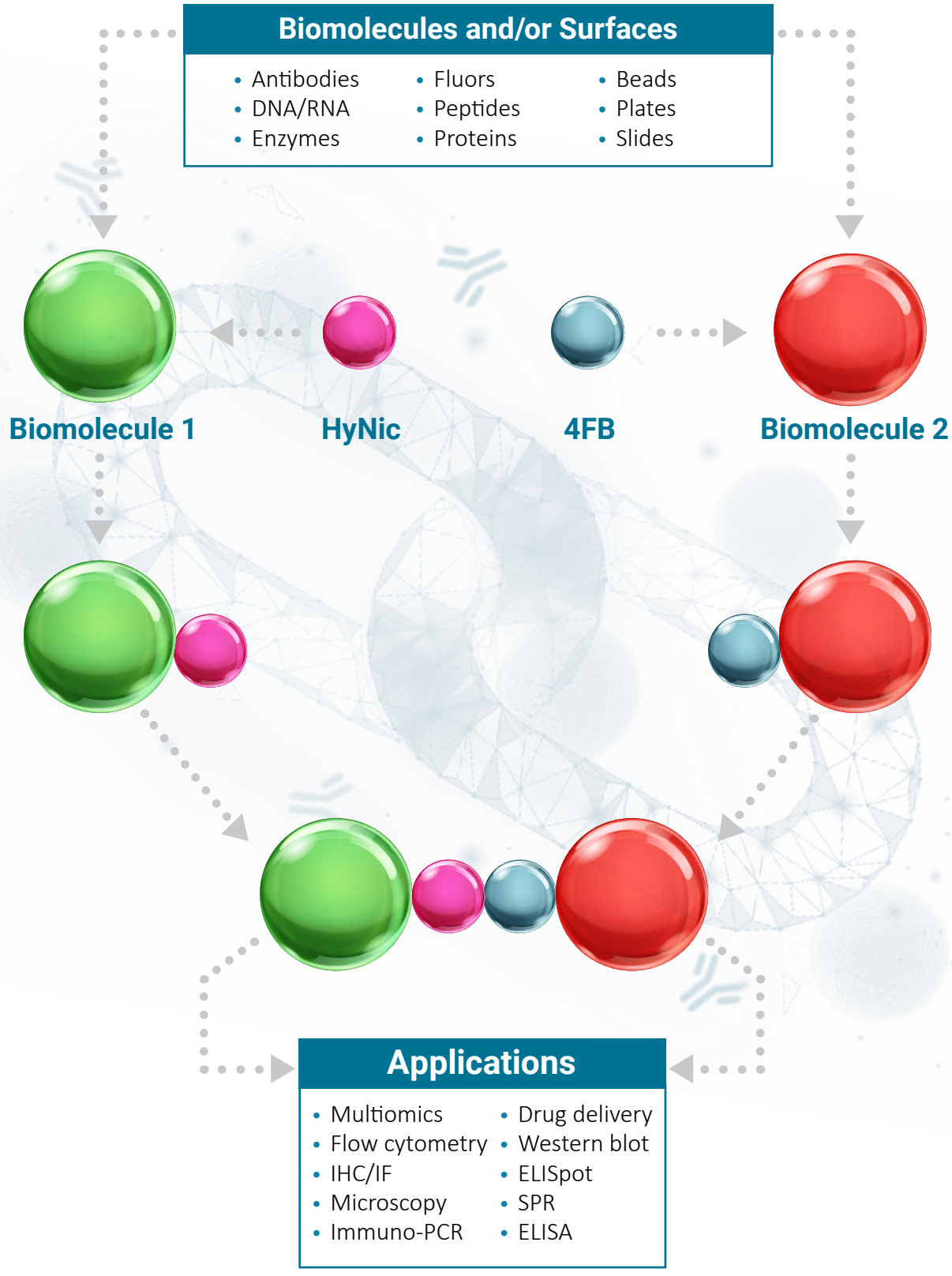
General bioconjugation workflow

What are the advantages of using SoluLINK bioconjugation technology?

SoluLINK bioconjugation technology provides numerous advantages over traditional bioconjugation methods. These include superior bioconjugate stability in aqueous phases; faster conjugation reactions with increased labeling efficiency and lower reagent costs; and no requirement for heavy metal catalysts or reducing agents. Moreover, unlike traditional approaches, the bioconjugation reactions are not compromised by the formation of homodimers.

A major benefit of this bioconjugation technology is that the bis-arylhydrazone bond is chromophoric, absorbing maximally at 354 nm to provide a traceable readout that can be measured by spectrophotometry. This not only allows researchers to quantify the number of linkers on each biomolecule prior to conjugation but, in turn, enables the precise number of ligands attached to each biomolecule to be determined. The traceable readout also provides real time monitoring of the conjugation reaction and permits easy visualization during FPLC or HPLC purification to rapidly identify fractions containing the desired conjugate.

Applications of SoluLINK Bioconjugation Technology



Uses and Advantages of Bioconjugates

Conjugated biomolecules are used in many different research applications. These include:

- Western blotting
- ELISA
- Immunocytochemistry (ICC)
- Immunohistochemistry (IHC)
- Flow cytometry
- Immuno-PCR

Depending on the workflow, biomolecules like antibodies, proteins or oligonucleotides may be conjugated to one another, labeled with haptens such as biotin, digoxigenin, fluorescein, R-phycoerythrin (R-PE), or allophycocyanin (APC), or bound to enzymes like horseradish peroxidase (HRP) or alkaline phosphatase (AP).

There are numerous advantages to using conjugated biomolecules for scientific research. Where antibodies are directly labeled with enzymes or fluorophores, the elimination of a secondary antibody incubation step shortens immunostaining workflows and can allow the number of parallel readouts to readily be increased, while antibodies labeled with oligonucleotides offer enhanced immunoassay sensitivity and a wider dynamic range compared to established techniques such as ELISA. Protein-protein conjugates also have broad utility, for instance as immunogens during antibody development where a large carrier protein may be attached to a smaller biomolecule, or as tools used to develop diagnostic tests like lateral flow assays.

Our product portfolio comprises kits for labeling biomolecules with:

- Haptens (biotin, digoxigenin, fluorophores)
- Oligonucleotides
- Enyzmes
- Proteins

We also offer a wide selection of products for customized conjugations, including S-HyNic and S-4FB crosslinkers for quick and easy amine functionalization. These are complemented by various conjugation accessory products to streamline your research, such as 2-Sulfobenzaldehyde and 2-Hydrazinopyridine.dihydrochloride for quantifying HyNic and 4FB biomolecule modification.

Biotin and Digoxigenin Labeling Kits

Incorporating measurable biotin or digoxigenin labels on antibodies and proteins maintains uniformity and performance characteristics between lots

- **Reproducible results—**UV-traceable chromophore permits nondestructive, rapid A_{280}/A_{354} quantification of incorporated biotin or digoxigenin, enabling consistency and reproducibility
- **Extended PEG3 spacer—**helps reduce aggregation, minimizes steric hindrance, and enhances solubility
- **Combine labeling technology—**to extend multiplex IHC staining capability

Biotin and Digoxigenin Applications

- Enables multiplex IHC/IFC
- Next-gen sequencing target enrichment
- ELISA, IHC, and IF assay development
- IVD immunoassay development

Quantitative measurement of bound biotin

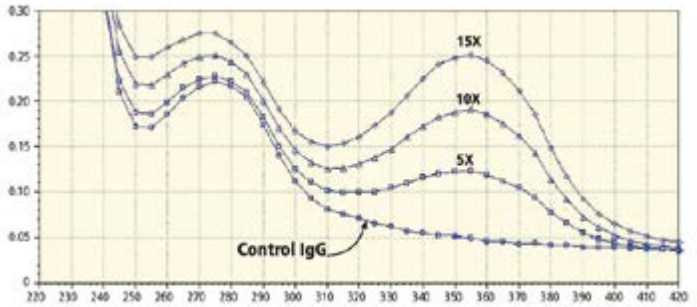


Figure 2. Superimposed spectra of desalted bovine IgG that was biotinylated using ChromaLINK Biotin at various biotin to protein mole equivalents (5X, 10X, and 15X).

ChromaLINK® Biotin or Digoxigenin contains a UV-traceable chromophore (Figure 1), based on SoluLINK bioconjugation technology, to enable reproducibility in your labeling process. Now you can measure the degree of biotinylation in minutes, not hours, without the standard curves required for HABA/avidin and fluoro-reporter assays. With a simple and direct UV scan, you can quantify biotin incorporation and ensure reproducible production of consistent batches (Figure 2).

Pair ChromaLINK Biotin Labeling with NanoLINK Streptavidin Magnetic Beads (see pages 19-21) for many types of assay development.

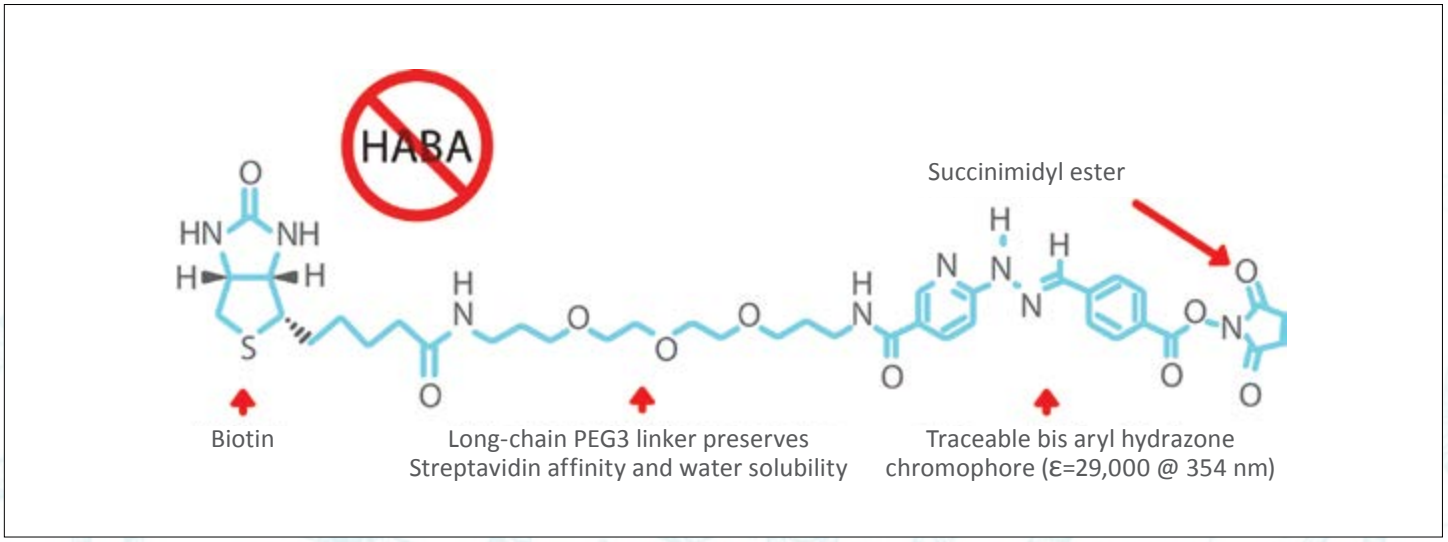


Figure 1. ChromaLINK Biotin.

ChromaLINK Biotin Labeling Kits

Depending on the actual material intended to be biotinylated, we offer two ChromaLINK biotin labeling Kits:

The ChromaLINK Biotin Protein Labeling Kit has all the necessary reagents for the traceable biotinylation of any lysine-containing protein. This kit provides sufficient materials to biotinylate and purify up to 5 proteins in about 2 hours. Each labeling reaction can be scaled from 25 µg to as much as 1 milligram of protein.

The ChromaLINK Biotin One-Shot™ Antibody-Labeling Kit is a simple, cost-effective way of incorporating a verifiable amount of biotin into a single 100 µg quantity of antibody. This kit can be used to label a variety of different antibodies including mammalian IgG (monoclonal or polyclonal) and avian IgY. Approximately 3-8 biotin molecules are incorporated per antibody that is easily determined using a non-destructive UV scan (220-400 nm) of the sample after labeling.

For investigators that require separate biotin linkers to perform custom conjugation, we offer several options. The linkers listed below all include the UV-traceable chromophore. Selection should be based on the specific biomolecule structure or sensitivity to organic solvent.

- Sulfo ChromaLINK Biotin (water soluble)
- ChromaLINK Biotin (DMF Soluble)
- ChromaLINK Biotin Maleimide

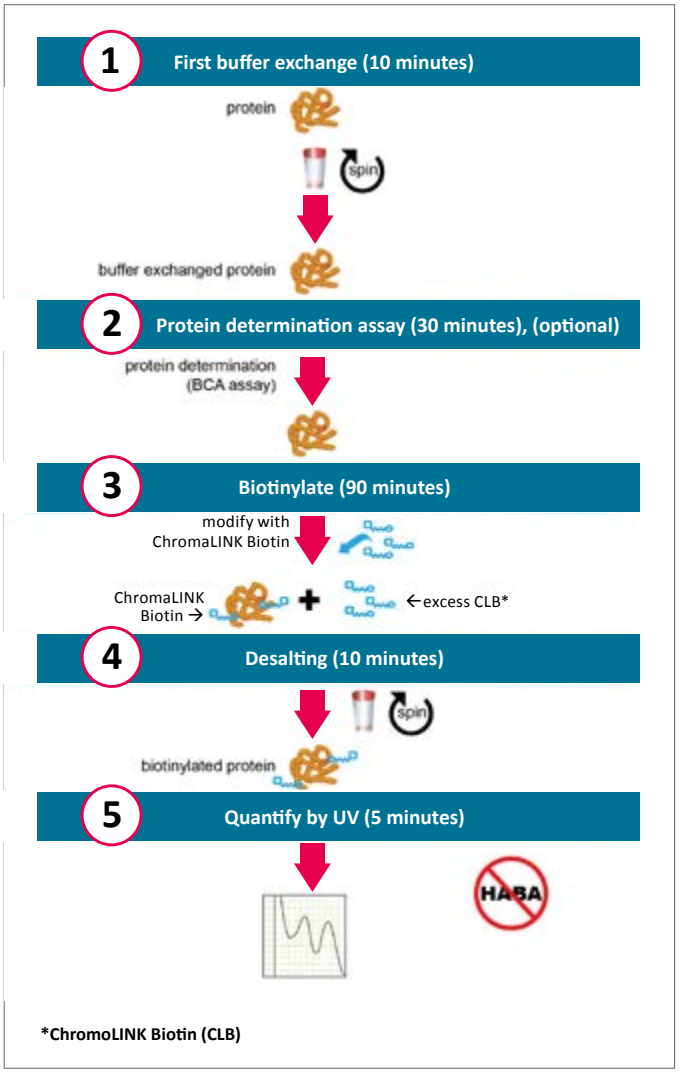


Figure 3. ChromaLINK Biotin Labeling Kit workflow.

Table 1. ChromaLINK Biotin Protein Labeling Kit out performs the competition

	ChromaLINK Biotin Protein Labeling Kit	Pierce EZ-LINK Sulfo-NHS and Biotinylation Kit
Biotinylation Time	2.5 hours	2.5 hours
Quantification of Biotin	5 minute UV Scan	3 hour HABA Assay

Free White Paper Download

How to Biotinylate with Reproducible Results

Describes limitations of traditional biotinylation reagents and explains how ChromaLINK Biotin overcomes these to enable fast, easy quantitation of biotinylation.

Download the paper at **vectorlabs.com/resources/brochures**

The first measurable Digoxigenin Labeling Kit

The ChromaLINK Digoxigenin One-Shot Antibody Labeling Kit provides convenient, consistent, and measurable digoxigenin labeling of 100 µg of antibody (Figure 4). Each kit contains ChromaLINK Digoxigenin, which incorporates a novel UV-traceable chromophore in the linker arm to enable reproducibility in your antibody labeling process. With a simple, non-destructive UV scan you can now quantify digoxigenin labeling to ensure reproducible incorporation of the optimal number of haptens per antibody. Each One-Shot kit contains everything needed to to label your antibody: buffers, reagents, desalting columns, and an easy-to-follow protocol and an online Digoxigenin incorporation calculator.

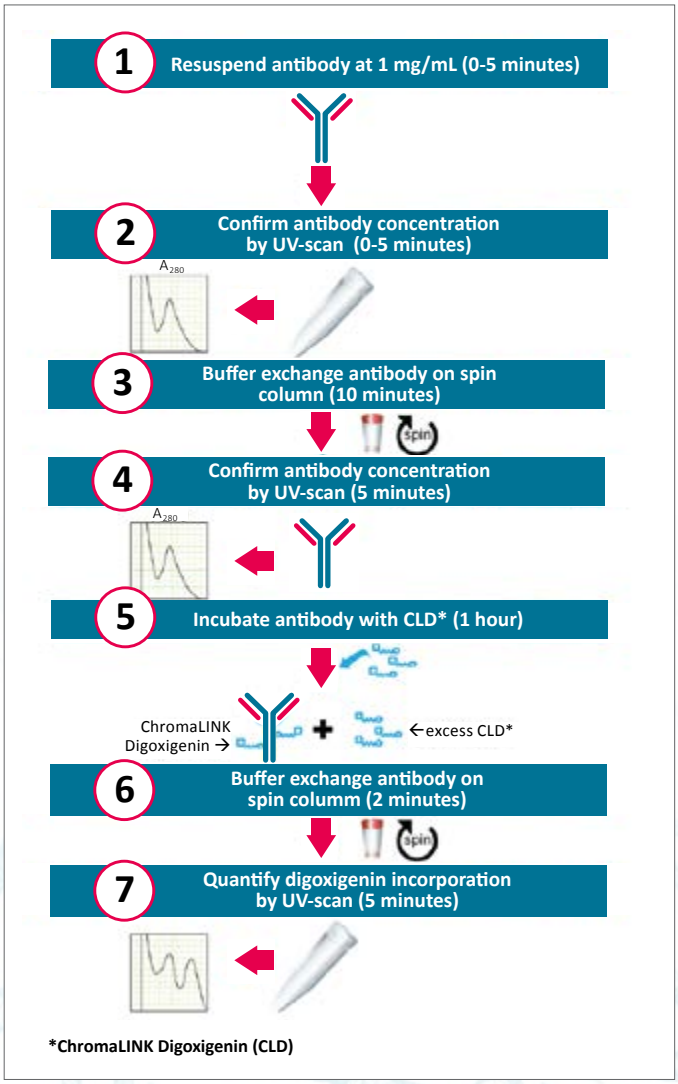


Figure 4. ChromaLINK Digoxigenin One-Shot Antibody Labeling Kit workflow.

Multiplex IHC Technique

Multiplexed immunodetection techniques such as immunofluorescence have traditionally been hampered by the relatively low number of antibodies available against cellular targets raised in different species. This limits the application of labeled secondary anti-species antibodies in a single tissue or cell sample. Remarkably, with the use of ChromaLINK Biotin and ChromaLINK Digoxigenin, same species primary antibodies may be labeled with haptens and subsequently detected with streptavidin and anti-digoxigenin antibody fluorescent conjugates, respectively, on the same sample without cross-reactivity. Additionally, since the primary antibodies contain multiple haptens for binding of labeled detector molecules, the signal is greatly enhanced when compared to directly labeled primary antibodies. Figure 5 shows the use of ChromaLINK Biotin and Digoxigenin-modified antibodies in a multiplexed immunofluorescence staining experiment using the same host species primary antibodies (mouse).

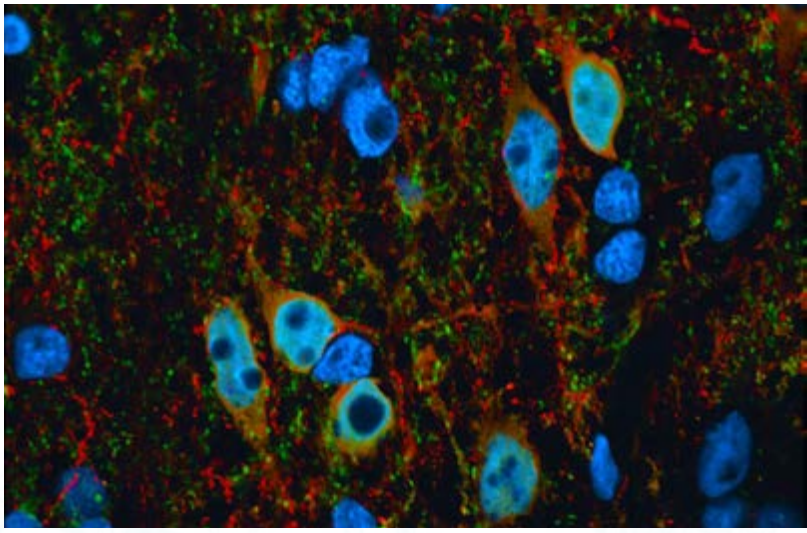


Figure 5. ChromaLINK Biotin and Digoxigenin-modified antibodies in a multiplexed immunofluorescence staining experiment using the same host species primary antibodies from the same host species (mouse).

Ordering Information

Product	Size	Cat. No.
Biotin		
ChromaLINK Biotin Protein Labeling Kit	Kit – Five reactions of 25 µg to 1 mg	B-9007-105
ChromaLINK One-Shot Antibody Biotinylation Kit	Kit – Labels 100 µg of Ab	B-9007-009
Sulfo ChromaLINK Biotin (Water Soluble)	10 mg	B-1007-110
	5 × 1.0 mg	B-1007-105
ChromaLINK Biotin (DMF Soluble)	10 mg	B-1001-010
	5 × 1.0 mg	B-1001-105
ChromaLINK Biotin Malemide	10 mg	B-1012-010
Digoxigenin		
ChromaLINK Digoxigenin One-Shot Antibody Labeling Kit	Kit – Labels 100 µg of Ab	B-9014-009

Selected Published References

- B-9007-105 (Ref. Nos. 1-3)
- B-9007-009 (Ref. Nos. 4-6)
- B-1007 (Ref. Nos. 7-10)
- B-1001 (Ref. Nos. 11-15)
- B-1012-010 (Ref. No. 16)
- B-9014-009 (Ref. No. 17)

Enzyme and Fluorophore Labeling Kits

While labeling antibodies and proteins with biotin and digoxigenin provides functionality, an alternative approach is to directly conjugate these biomolecules with an enzyme or fluorophore. SoluLINK bioconjugation technology offers the following advantages when considering this approach:

- No time consuming chromatography required
- **Fast conjugations—**fast catalyzed method generates conjugates in approximately 4–6 hours
- **Efficient—**100% conversion with 40–70% yields

The enzyme and fluorophore labeling kits (Figure 6) offer an innovative, efficient, and easy-to-use method based on the SoluLINK bioconjugation technology. They deliver pure, and ready-to-use direct-labeled conjugates eliminating the need for lengthy FPLC or HPLC, so you can focus on downstream applications.

Antibody Labeling Applications

- Flow cytometry assay development
- Western blot
- Immunofluorescence staining

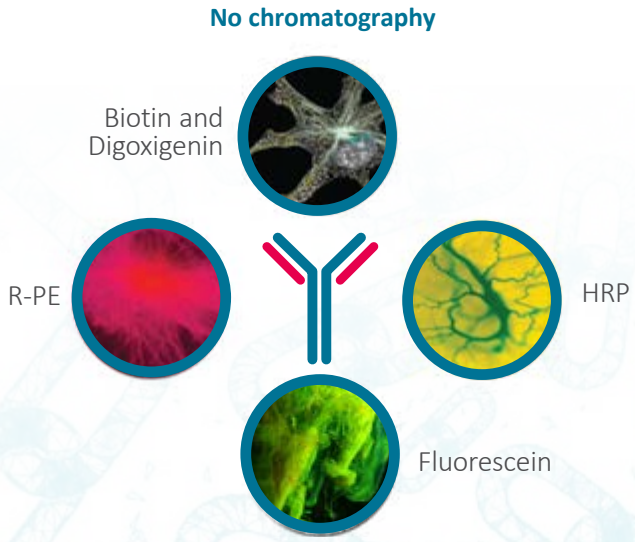


Figure 6. Labels available for antibody labeling with the SoluLINK bioconjugation technology.

How it works

This technology is based on the use of two complementary heterobifunctional linkers:

- **S-HyNic** (succinimidyl-6-hydrazino-nicotinamide) linker, an NHS ester, reacts with lysine residues, incorporating HyNic functional groups (hydrazinonicotinamide) onto the antibody.
- **S-4FB** (succinimidyl-4-formylbenzamide) linker is conjugated to the label, providing a pre-activated, high-activity label (example, 4FB-HRP).

HyNic-modified antibody is incubated with pre-activated 4FB label (example, 4FB-HRP) leading to rapid and efficient conversion of the antibody to conjugate through formation of stable bis-arylhydrazone bonds (Figure 7).

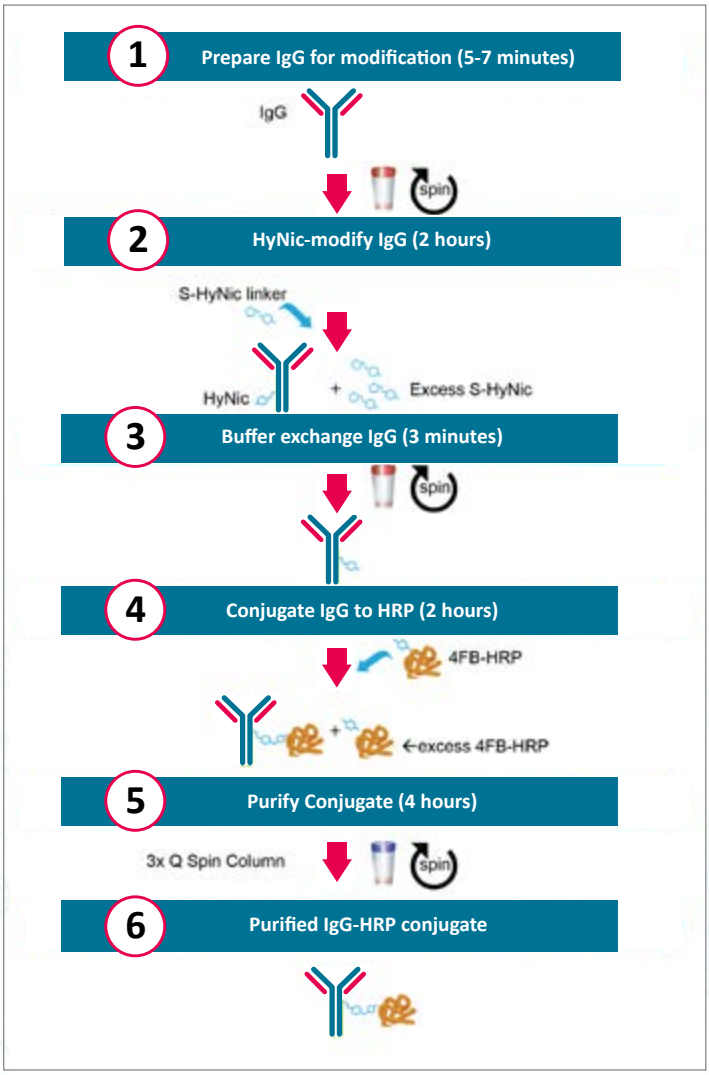


Figure 7. HRP-Antibody All-in-One Conjugation Kit workflow.

Faster and complete conjugations

The comprehensive HRP-Antibody All-in-One™ Conjugation Kit incorporates TurboLINK™ catalyst, aniline, into the linking technology, delivering 100% conversion of antibody to conjugate. The addition of aniline as a catalyst using this linking chemistry has been previously described (Dirksen, A. and Dawson. P.E. *Bioconjugate Chem.* 2008, 19(12):2543-2548).

This reaction takes place under mild conditions and increases the rate and efficiency of the labeling reaction, leading to quantitative conversion of free antibody to conjugate. The complete absence of free antibody at the end of the catalyzed reaction leaves only two components in excess: **Label and Conjugate**.

The HRP Antibody All-in-One Conjugation Kit offers a high-yield purification method without HPLC. After conjugation, a novel Q spin filter is used that quantitatively removes excess HRP to provide high-purity, ready-to-use conjugate. Purified conjugate is then eluted from the filter membrane, free of residual antibody and label in high yield.

The R-PE-Antibody Conjugation Kit is designed for ultimate flexibility and will conjugate two reactions of 150 µg to 1.3 mg of any user-supplied antibody with pre-activated R-Phycoerythrin. Any suitably pure monoclonal or polyclonal antibody can be conjugated to R-PE and purified in just over 4 hours.

This product features high-fluorescent R-PE, efficient SoluLINK chemistry, and a flexible kit platform that allows you to adjust labeling and amounts of antibody used in the conjugation.

The Fluorescein Antibody Labeling Kit is designed to label a microscale quantity of antibody (100 µg) with 3 to 5 fluorescein molecules per antibody. The kit contains sufficient reagents to perform two labeling reactions, 100 µg of antibody per reaction. Fluorescein-labeled antibodies can be used for standard immunofluorescent staining and imaging of cells or tissues. This kit contains all the necessary components to label and purify an antibody in about 90 minutes.

Ordering Information

Product	Size	Cat. No.
HRP		
HRP-Antibody All-in-One Conjugation Kit	Kit – conjugates 2 × 100 µg Ab	A-9002-001
R-PE, and Fluorescein		
R-PE-Antibody Conjugation Kit	Kit – conjugates 2 × 150 µg to 1.3 mg Ab	P-9002-002
Fluorescein One-Shot Antibody Labeling Kit	Kit – labels 2 × 100 µg of Ab	F-9001-009K

Selected Published References

- A-9002-001 (Ref. No. 18)
- P-9002-002 (Ref. No. 19)

Free White Paper Download

How to Prepare Quantifiable HRP Conjugates

Limitations of traditional linker technologies and the advantages of using the catalyzed linker technology to prepare highly pure protein poly-HRP complexes.

Download the paper at **vectorlabs.com/resources/brochures**

Oligonucleotide Labeling

Traditional methods of labeling oligonucleotides with antibodies or proteins have relied on maleimide-thiol based chemistry and other involved methods that compromise reproducibility and efficiency. Using the SoluLINK chemistry simplifies the process and generates high-yielding conjugates.

Antibody-Oligonucleotide All-in-One Conjugation Kit

Each kit provides all the necessary reagents to generate one antibody-oligonucleotide conjugate. The kit requires the user to supply the antibody (polyclonal or monoclonal mammalian IgG) and one HPLC-purified, 3' or 5' amino-modified oligonucleotide. Typically, a 1 µmol synthesis provides sufficient amino oligo for modification. Kit instructions are specifically designed for researchers with limited or no conjugation experience. A specific conjugation calculator is directly integrated with the protocol and avoids the need to perform numerical calculations throughout the procedure. Each kit yields between 30–50 µg of highly purified, ready-to-use, antibody-oligonucleotide conjugate.

- **High yield—**30–50% yield based on starting antibody
- **High purity—**>95% purity without chromatographic purification
- **High stability—**conjugates are stable for >1 year

Antibody-Oligonucleotide Applications

- Immuno-PCR
- High-sensitivity protein detection
- Antibody arrays

Conjugates produced with the Antibody-Oligonucleotide All-in-One Kit are ready to be used in the most demanding and sensitive of downstream applications. The kit delivers high-purity conjugate virtually free of residual antibody or oligonucleotide (>98%). Reaction conditions are optimized to convert nearly 100% of the antibody into conjugate, leaving only free, excess 4FB-oligo to be removed. Complete conversion of antibody to conjugate simplifies conjugate purification as illustrated (Figure 8).

Antibody-oligonucleotide conjugate is purified to near homogeneity by selectively binding the conjugate to a magnetic affinity matrix, allowing excess 4FB-oligonucleotide to be washed away. Affinity-bound conjugate is then gently eluted from the matrix and buffer exchanged into long-term storage buffer.

Antibody-oligonucleotide conjugates produced with this kit are stable for up to 1 year when kept at 4°C in storage buffer.

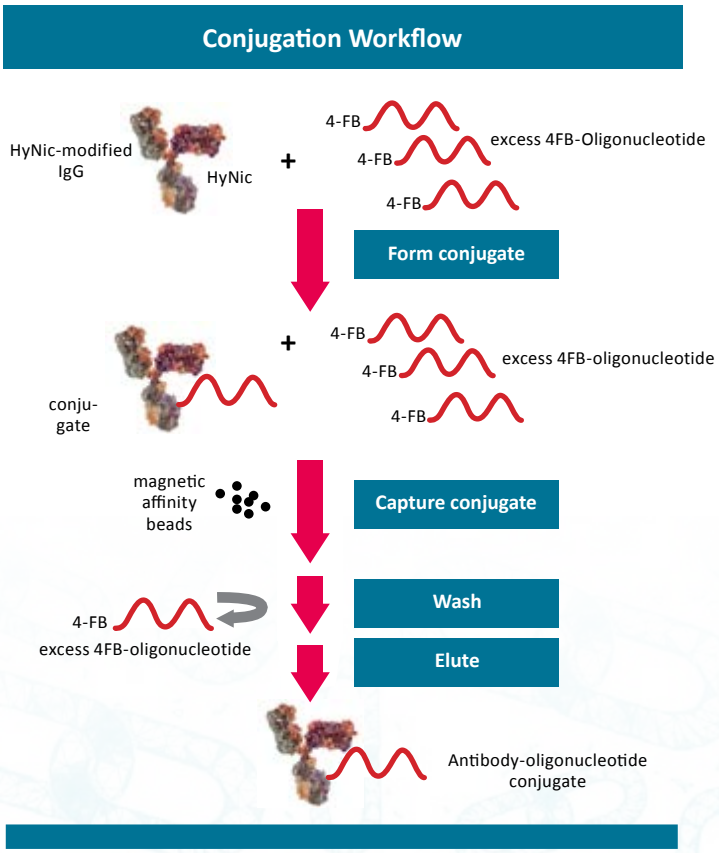


Figure 8. The Antibody-Oligonucleotide All-in-One Conjugation Kit workflow

The Protein-Oligonucleotide Conjugation Kit

The Protein-Oligonucleotide Conjugation Kit is designed to conjugate a protein with an oligonucleotide. It includes all of the necessary components and protocols for easy and specific crosslinking of any protein with any amino-oligo from 20 to 100 bases in length. This kit is flexible so that researchers with little or no conjugation experience can make their own custom protein-oligonucleotide conjugate to suit their needs.

The Protein-Oligonucleotide Conjugation Kit uses the SoluLINK chemistry to prepare protein-oligonucleotide conjugates in 3 easy-to-perform steps (Figure 9). The first step is the modification of the oligonucleotide with the 4FB crosslinker, followed by the formation of the HyNic modified protein. Finally, simple mixing of the two modified biomolecules will result in the formation of a stable, UV-traceable bond formed by the reaction of a HyNic modified protein with a 4FB modified oligonucleotide.

This technology has many practical advantages compared to traditional crosslinking methods:

- The reaction is high yielding. Routine yields of conjugate are 50-80% based on starting protein.
- The reaction is efficient: Only 3-4 mole equivalents of oligonucleotide are necessary for the protein, >95% of the protein is conjugated.
- The conjugate bond is extremely stable: The conjugate bond is stable to 92°C and pH 2.0-10.0.
- The reaction conditions are mild and do not cause any protein denaturation. No metals, oxidation or reducing reagents are required.
- The conjugation is traceable spectrophotometrically.
- The modifications of both the HyNic linker on the protein and the 4FB linker on the oligonucleotide are quantifiable using colorimetric assays.

Ordering Information

Product	Size	Cat. No.
Antibody-Oligonucleotide All-in-One Conjugation Kit	Kit–conjugates 100 µg of antibody	A-9202-001
Protein-Oligonucleotide Conjugation Kit	Kit – 2 reactions of 50 – 650 µg of protein, each	S-9011-1

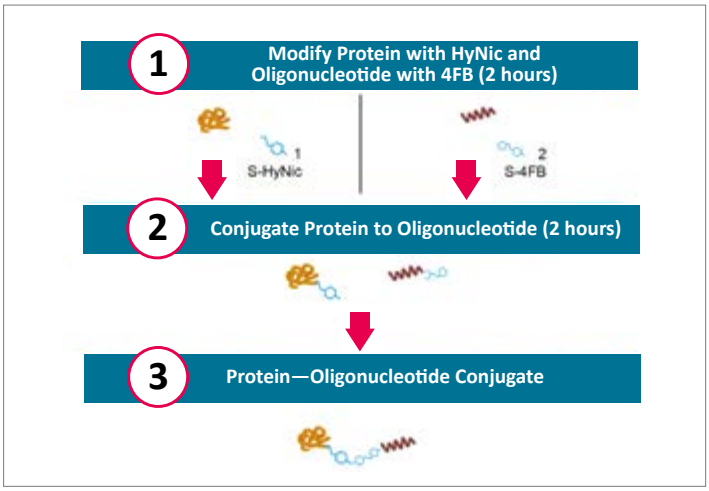


Figure 9. Protein-Oligonucleotide Conjugation Kit workflow.

Selected Published References

- A-9202-001 (Ref. Nos. 20-22)
- S-9011-1 (Ref. Nos. 23-25)

Free White Paper Download

Antibody-Oligonucleotide Conjugate Preparation

This white paper explains how to prepare Antibody-oligonucleotide conjugates efficiently in high yields and at high purity without chromatography.

Download the paper at **vectorlabs.com/resources/brochures**

Protein-Protein Labeling

The Protein-Protein Conjugation Kit

This kit is designed to conjugate two (2) reactions, each using 50-650 µg of each protein 20kDa or greater with any other protein of equal or greater size. Any suitably pure monoclonal or polyclonal antibody can be conjugated as well as any other amine containing proteins. The kit utilizes the HyNic/4FB coupling to produce these high quality protein conjugates with high yield (Figure 10). Common examples of protein-protein conjugates produced using this kit include HRP-antibody and PE-antibody. This kit is flexible so that researchers with little or no conjugation experience can make their own custom protein-protein conjugates to suit their needs.

The protein-protein conjugates generated with this kit may be used for applications including ELISA, flow cytometry, microarray-based immunoassays, immunofluorescence and immunohistochemistry. Conjugates can be used for these and other applications where high quality conjugates are required.

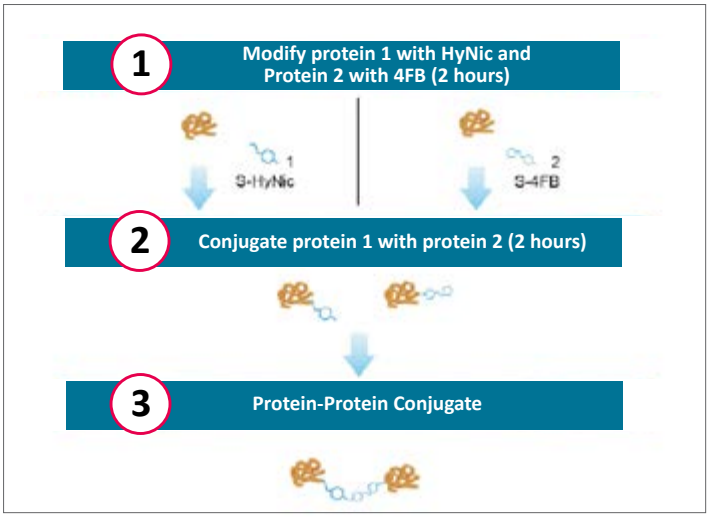


Figure 10. Protein-Protein Conjugation Kit workflow.

Ordering Information

Product	Size	Cat. No.
Protein-Protein Conjugation Kit	Kit	S-9010-1

Free White Paper Download

Quantitative and Reproducible Bioconjugation with SoluLINK Technology
This white paper explains the benefits of using catalyzed linker technology to conjugate biomolecules to each other or to surfaces.

Download the paper at
vectorlabs.com/resources/brochures

Selected Published References

- S-9010-1 (Ref. Nos. 26-29)

Conjugation Accessories

Time-saving components to help you reach your ultimate research goals...quickly

Most conjugation kit components can be purchased separately as stand alone reagents. These products include TurboLINK, modification buffer, conjugation buffer, spin filters, desalting columns, and quenching reagents. These separate components can be used to supplement reagents supplied in a given kit format, or can be used in combination with separate linkers (see p. 16) to complete a conjugation workflow of a new or novel compound.

Ordering Information

Product	Size	Cat No.
TurboLINK Catalyst Buffer	1.5 mL	S-2006-105
Conjugation Buffer (10X)	5 × 1.5 mL	S-4002-005
Modification Buffer (10X)	5 × 1.5 mL	S-4000-005
Zeba Desalting Columns	10 pack	S-4024-010
Anhydrous DMF	5 × 1.5 mL	S-4001-005
2- Sulfobenzaldehyde	100 mg	S-2005-100
2- Hydrazinopyridine.dihydrochloride	100 mg	S-2002-100

Selected Published References

- S-2006-105 (Ref. No. 30)
- S-4002-005 (Ref. Nos. 31-33)
- S-4000-005 (Ref. Nos. 34-36)
- S-4001-005 (Ref. Nos. 37-39)
- S-2005-100 (Ref. Nos. 40-41)
- S-2002-100 (Ref. Nos. 42-46)

Separate Linkers for Conjugation

Linkers for all biomolecules

The conjugation kits described in this guide provide straightforward and easy solutions to accomplish a lot of standard assays. The kit formats are recommended for investigators that may be new to the field of bioconjugation, or for investigators with a fairly routine conjugation requirement.

Many labs, however, have needs that extend beyond what the kit formats provide. In these instances, separate linkers are available to accommodate more advanced bioconjugation needs. The separate linkers use the same SoluLINK bioconjugation technology incorporated in the kit formats.

Using separate HyNic and 4FB linkers, any two biomolecules, regardless of molecular weight, can be conjugated efficiently. Mixing of the two biomolecules, with TurboLINK catalyst, allows the two linkers to rapidly, selectively, and efficiently react with each other. The result is two biomolecules conjugated through a UV-traceable, stable bond (bis-arylhydrazone) with measurable absorbance at 354 nm. These linkers are available as reagents or bead products to enable next-generation biomedical assays and detection systems.

Projects that may require separate linkers include:

- Immunoassay development
- Sample preparation
- Increasing functionality of new or novel compounds

Using separate linkers for your project enables:

- **Fast conjugations—**TurboLINK catalyst means faster kinetics for higher efficiency and yields
- **Efficient—**>95% efficient linker-biomolecule conjugations
- **Stable and robust—**conjugate bond is stable to 92˚C and pH 2.0–10.0

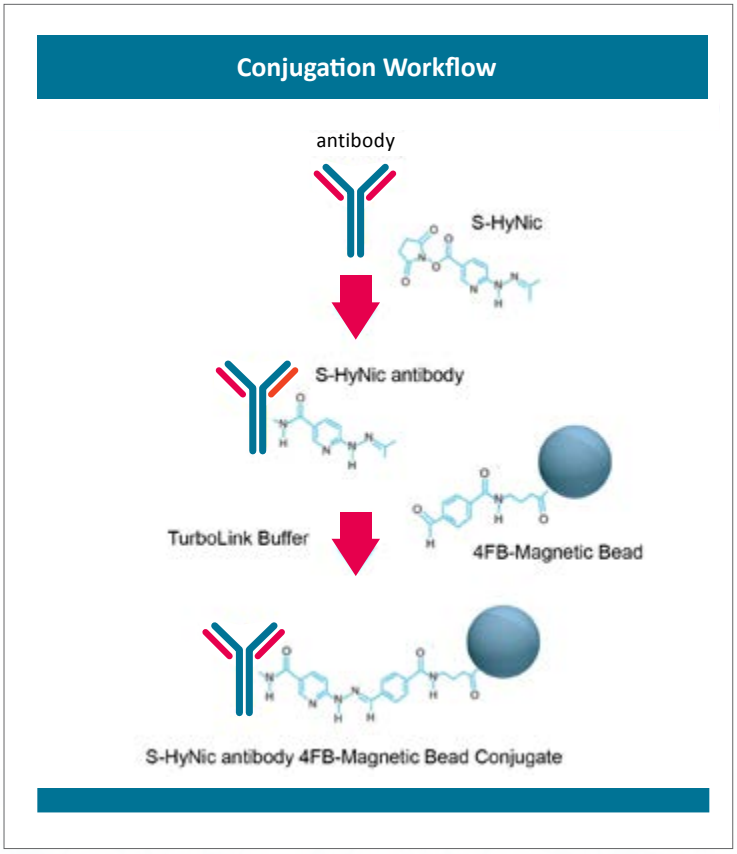


Figure 11. Use of separate linker to perform novel conjugation.

Selected Published References

- S-1002 (Ref. Nos. 46-51)
- S-1011-010 (Ref. No. 52)
- S-1004 (Ref. Nos. 53-58)
- S-1008 (Ref. Nos. 23-25, and 59)
- S-1001-010 (Ref. Nos. 60-63)
- S-1009-010 (Ref. Nos. 30, 42, and 64-66)

Table 2. Linker selection guide

A – Type of conjugate	B – Type of molecule	C – Reactive group		D – product
Antibody-Protein	Ab	If using an Amino (NH2) then use:	Linker 1	S-HyNic [S-1002-105]
		If using a Thiol (SH2) then use:		MHPH [S-1009-010]
	Protein	If using an Amino (NH2) then use:	Linker 2	S-4FB [S-1004-105]
Protein-Oligo	Protein	If using an Amino (NH2) then use:	Linker 1	S-HyNic [S-1002-105]
		If using a Thiol (SH2) then use:		MHPH [S-1009-010]
	Oligo	If using a 3' or 5' Amino, then use:	Linker 2	S-4FB [S-1004-105]
Protein-MagnaLINK Beads conjugation	MagnaLINK Beads	If using 4FB MagnaLINK Beads, then use:	Linker 1	4FB MagnaLINK Beads [M-1004-010]
	Ab, other protein, R-PE, APC, perCP, HRP, AlkPhos	If using an Amino (NH2) then use	Linker 2	S-HyNic [S-1002-105]
		If using a Thiol (SH2) then use:		MHPH [S-1009-010]

Instructions: This technology requires two linkers to successfully conjugate 2 biomolecules to give you a quantifiable, controllable and stable result.

Step 1: Select type of conjugate (A)

Step 2: Select the 1st biomolecule (B) then select its reactive group (C) The product and catalog number needed appears in the same row in column D. (LINKER 1)

Step 3: Select the 2nd biomolecule (B) then select its reactive group (C) The product and catalog number needed appears in the same row in column D. (LINKER 2)

Step 4: For successful conjugation, order the products referenced in steps 2 and 3 (column D).

Ordering Information

Product	Size	Cat. No.
HyNic		
S-HyNic LInker (DMF Soluble)	5 × 1.0 mg	S-1002-105
	10 mg	S-1002-010
Sulfo S-HyNic Linker (Water Soluble)	10 mg	S-1011-010
S-4FB Linker (DMF Soluble)	5 × 1.0 mg	S-1004-105
	10 mg	S-1004-010
Sulfo-S-4FB Linker (Water Soluble)	5 × 1.0 mg	S-1008-105
	10 mg	S-1008-010
S-SS-4FB Cleavable Linker	10 mg	S-1037-010
Surface Linkers and other linkers		
SHNH (HyNic for Technetium Labeling)	10 mg	S-1001-010
MHPH (Maleimide HyNic) Linker	10 mg	S-1009-010

Biomolecule Capture

Bead-based capture of target analytes from solution is employed for many different research applications. Typically, magnetic or agarose beads are used, where the beads are labeled to enable capture of a complementary binding partner; this can then be extracted from solution by magnetic separation or centrifugation.

For bead-based capture to be effective, it is important that the beads are monodispersed in solution and of a uniform size to ensure reproducibility from one experiment to the next. Beads should also have a large surface area and high binding capacity to maximize target capture and should be stable both in colloidal form and in a diverse range of sample matrices. Magnetic beads should additionally exhibit a rapid magnetic response time and should have no exposed iron that can be incompatible with certain buffer components, whereas agarose beads should have no net charge and should be tolerant of the high pressures and centrifugal forces often experienced in high throughput screening and purification applications.

Vector Laboratories offers a broad selection of magnetic and agarose beads, all of which are produced using SoluLINK bioconjugation technology for consistent, high-capacity target binding. This bioconjugation technology provides improved bioconjugate stability compared to traditional bioconjugation methods, and it also benefits from faster conjugation reactions with increased labeling efficiency as well as enabling easy quantification of linker incorporation for unparalleled reproducibility in conjugate formation.

Two core magnetic bead sizes are available to suit a variety of applications, including NanoLINK (1 µm) streptavidin and MagnaLINK (2.8 µm) streptavidin and 4FB formats, while our Streptavidin Agarose complements these to further increase the scope of your research. The high surface area of all our beads, combined with the efficiency of SoluLINK bioconjugation, translates to lower bead requirements and proportionally lower backgrounds and cost. For example, both NanoLINK and MagnaLINK Streptavidin Magnetic Beads possess up to 15-times greater biotin binding capacity than other commercially available products, while our Streptavidin Agarose demonstrates the highest biotin binding capacity of any agarose bead on the market.

NanoLINK®, MagnaLINK® and Streptavidin Agarose

Capture your biontinylated biomolecule

Streptavidin magnetic beads and streptavidin agarose are offered with the highest biotin binding capacity on the market—beads with as much as 15X higher binding capacity and agarose at a 20% lower price than competing products. Higher binding translates into reduced bead mass or agarose required to immobilize a biotinylated sample and lower background noise from nonspecific binding, resulting in better signals and lower net costs.

- **Highest biotin binding—**enabled by unique streptavidin crosslinking
- **Fast (<2 min) response time—**saves time and accommodates viscous samples
- **Versatile—**ideal for a variety of applications

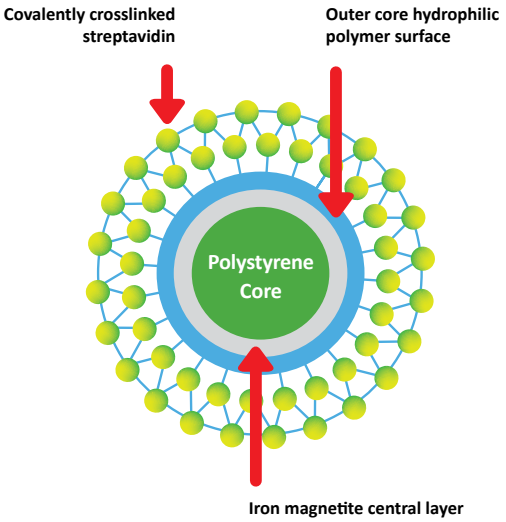


Figure 12. Illustration showing cross section of NanoLINK and MagnaLINK Streptavidin Magnetic Beads.

Magnetic Bead and Agarose Applications

- Antibody-based cell separation
- IVD immunoassay development
- ChIP and DNA/RNA binding protein isolation
- Immunoprecipitation and protein isolation
- Next-gen sequencing target enrichment

The secret is in the crosslinking

NanoLINK 1.0 micron and MagnaLINK 2.8 micron magnetic beads are super-paramagnetic, hydrophilic polymer—encapsulated (no exposed iron), monodispersed microspheres with a fast (<2 minutes) magnetic response time. They are stable in colloidal form and in detergents. The key to high biotin binding is in the unique covalently crosslinked streptavidin, based on SoluLINK bioconjugation technology (Figure 12). The high surface area, when combined with our efficient linking chemistry, produces a consistent, ultra-high biotin binding bead (Table 3).

Table 3. NanoLINK and MagnaLINK binding capacity

Molecule	NanoLINK (1.0 µm) binding capacity	MagnaLINK (2.8 µm) binding capacity
Free biotin	>12 nmol/mg	>10 nmol/mg
Biotinylated oligo (23-mer)	>2.5 nmol/mg	>0.8 nmol/mg
Biotinylated IgG (3 biotins per IgG)	>250 µg/mg	>112.6 µg/mg

NanoLINK, MagnaLINK & Streptavidin Agarose (continued)

NanoLINK Streptavidin Magnetic Beads

The beads are supplied at 1% solids (10 mg/mL) in nuclease-free water with 0.05% sodium azide. No surfactants are present.

Key features

- Highest free biotin binding capacity of any bead (>/= 12 nmol/mg). Refer to (Table 4)
- Binds ≥ 2.5 nmol/mg of a biotinylated oligonucleotide
- Binds ≥ 1.7 nmol/mg of a biotinylated-IgG (250 µg/mg) at 3 biotins/IgG
- Beads are encapsulated (no exposed iron)
- Beads are textured, providing increased surface area for binding
- Super-paramagnetic (no residual magnetism)
- Fast magnetic response time (<2 minutes)

NanoLINK beads are ideal for immobilizations and co-immunoprecipitation applications.

MagnaLINK Streptavidin Magnetic Beads

MagnaLINK 2.8 micron beads demonstrate exceptional size uniformity of <5% CV, evident by scanning electron microscopy (SEM) (Figure 14), which makes them ideal for high-throughput robotic applications.

MagnaLINK beads are supplied at 1% solids (10 mg/mL) in nuclease-free water with 0.05% sodium azide.

Key features

- Highest free biotin binding capacity of any uniform bead (≥ 10 nmol/mg)
- Binds 0.8 nmol/mg biotinylated oligonucleotide
- Binds 0.75 nmol/mg biotinylated-IgG at 4 biotins/IgG
- Beads are encapsulated (no exposed iron)
- Super-paramagnetic beads are highly uniform in size (2.8 +/- 0.2 microns)
- Fast magnetic response time (40% w/w magnetite)

Table 4. NanoLINK binding capacity outperforms the competition

Ligand	NanoLINK (1.0 µm) binding capacity	Competitor's (1 µm) binding capacity
Free biotin	>12,000 pmol/mg	>1,300 pmol/mg
Biotinylated oligo (23-mer)	>2.5 nmol/mg	NA
Biotinylated IgG (3 biotins per IgG)	>1.7 nmol/mg (250 µg/mg)	0.12 nmol/mg (20 µg/mg)

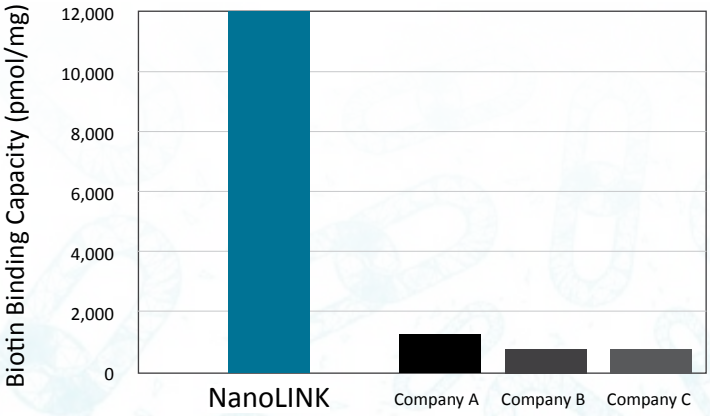


Figure 13. NanoLINK advantage: Competitive landscape of free biotin binding capacity.

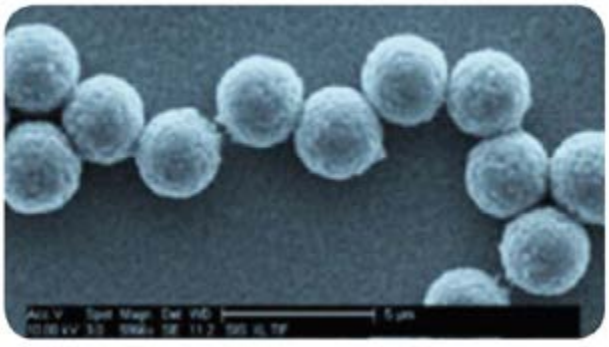


Figure 14. MagnaLINK Streptavidin Magnetic Beads have exceptional size uniformity.

Linker-activated magnetic beads

4FB magnetic beads provide a high surface area activated with 4FB linker to enable easy covalent immobilization for user-defined, high performance affinity purification schemes. The 4FB linker enables easy and efficient immobilization of any biomolecule premodified with the complementary HyNic linker.

Streptavidin Agarose

- **High binding capacity—**higher biotin binding capacity at >20% lower price
- **Crosslinked agarose—**The streptavidin agarose linker enables higher binding capacity, lower background, and less leaching
- **Multiple sizes—**available in 2 mL, 5 mL, 10 mL, and bulk quantities

Streptavidin Agarose Ultra Performance™ provides high biotin binding at a low price. The SoluLINK bioconjugation technology is coupled with a 6% highly crosslinked agarose to boost the biotin binding capacity of the high specific activity streptavidin. This ideal combination provides a biotin binding capacity of >330 nmol/mL of resin—one of the highest loading capacity products currently available (Figure 15). Use Streptavidin Agarose Ultra Performance for improved recovery of any biotinylated biomolecule to lower nonspecific binding, reduce costs, and produce better results.

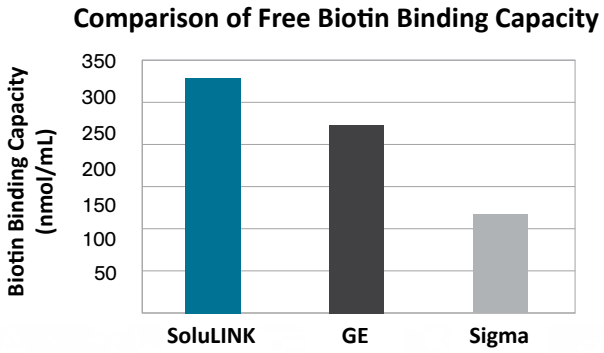


Figure 15. Comparison of streptavidin agarose free biotin binding capacity. SoluLINK binds 330 nmol/mL resin.

Ordering Information

Product	Size	Cat. No.
NanoLINK Streptavidin Magnetic Beads (1.0 µm)	1 mL at 10 mg/mL	M-1002-010
	2 mL at 10 mg/mL	M-1002-020
	5 mL at 10 mg/mL	M-1002-050
	10 mL at 10 mg/mL	M-1002-100
MagnaLINK Streptavidin Magnetic Beads (2.8 µm)	1 mL at 10 mg/mL	M-1003-010
	5 mL at 10 mg/mL	M-1003-050
	10 mL at 10 mg/mL	M-1003-100
4FB Magnetic Beads (2.8 µm)	1 mL at 10 mg/mL	M-1004-010
Streptavidin Agarose Ultra Performance	2 mL	N-1000-002
	5 mL	N-1000-005
	10 mL	N-1000-010

Selected Published References

- M-1002 (Ref. Nos. 67-71)
- M-1003 (Ref. Nos. 72-75)
- N-1000 (Ref. Nos. 76-78)

Free White Paper Download

Streptavidin Magnetic Beads to Optimize the Signal-to-Noise Ratio.
This white paper compares the NanoLINK and MagnaLINK streptavidin beads to competitor products.

Download the paper at **vectorlabs.com/resources/brochures**

Bioconjugation Custom Services/OEM/Bulk

While the bioconjugation kits and linking products that Vector Laboratories provides enable users to address many of their conjugation needs, sometimes researchers would rather outsource the work due to increased scale, need for enhanced analytical capabilities, or just so they can focus on other important elements of their work and leave the conjugate production to the experts where a kit is not available for a particular construct. When more material is needed or the job requires specialized equipment or expertise, Vector Laboratories' Custom Bioconjugation Services may be the answer.

Skilled technicians work in scales of micrograms to grams, using SoluLINK bioconjugation technology to connect antibodies, oligonucleotides, immunogenic and fluorescent proteins, peptides, surfaces, small molecules, and more. Using the HyNic and 4FB linkers, most biomolecules can be conjugated efficiently and reproducibly. As described in this resource guide, SoluLINK technology provides the means to quantitate the number of linkers on each conjugation partner and the efficiency of the conjugation itself. This provides an unparalleled level of control over the conjugation reaction and ensures the highest lot-to-lot reproducibility of your conjugate.

Once the large-scale conjugates are formed, a key next step is purification. Our labs are equipped to perform a number of different purifications based on size exclusion chromatography (SEC), cation- and anion-exchange, mixed-mode chromatography, hydrophobic interaction, reversed-phase chromatography and other methods which are capable of purifying grams of material as a single lot. While the conjugation reaction is efficient, the optimum mole ratio of components is often unbalanced to ensure that all of one of the components is conjugated, and the purification step is used to separate the conjugated material from unconjugated reactants.

HRP-antibody conjugate SEC purification chromatogram and heat map

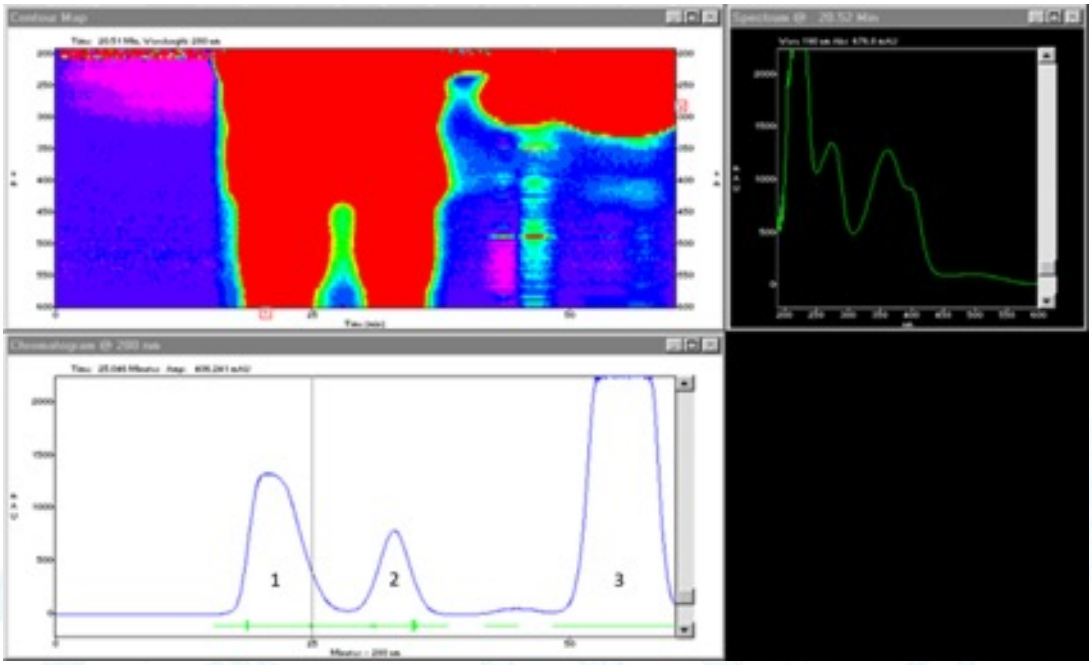
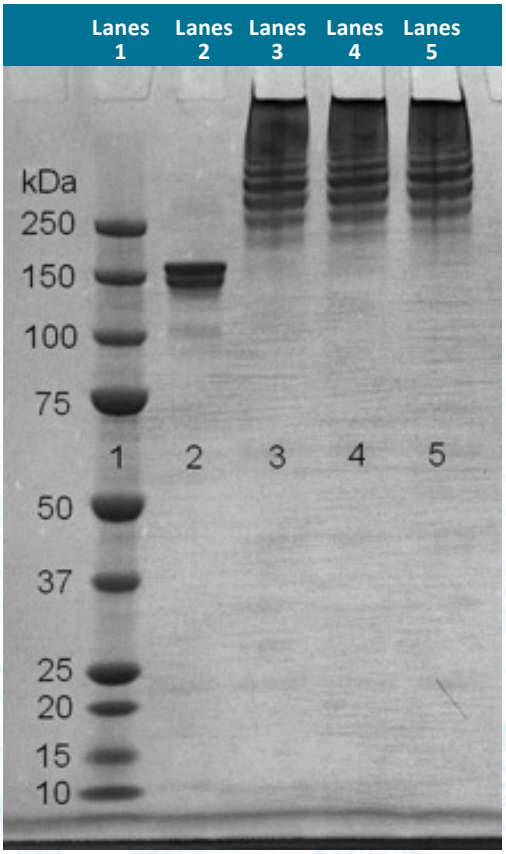


Figure 16. Size exclusion purification of a large-scale lot of HRP-antibody conjugate. The upper-left panel shows a heat map of absorbance across wavelengths vs. time, the upper-right panel displays the absorbance spectrum of the HRP-antibody conjugate at 25 minutes, and the lower-left panel shows the chromatogram at 280 nm. Peak 1 is the desired HRP-antibody conjugate, peak 2 consists of excess HRP, and peak 3 is 2-sulfobenzaldehyde (2-SB). Note the large absorbance at 350 nm in the upper-right spectrum due to the HyNic-4FB hydrazone bond. During conjugation, the reaction was quenched (stopped) by addition of 2-SB once the hydrazone 354 nm absorbance reached a determined value, indicating the desired degree of conjugation (molecular weight distribution) had been reached.

The conjugate peak can be clearly identified by the bis-arylhydrazone absorbance indicative of the 4FB and HyNic linkers joined together. For conjugation between two small molecule compounds, Liquid Chromatography-Mass Spectrometry (LC-MS) provides a means to detect and quantify the conjugated molecules directly. MALDI-TOF is used to determine the exact level of incorporation of haptens, drugs, and other small molecules onto larger, more complex biomolecules such as proteins in a bioconjugate sample.

An analytical SEC or ion exchange column run using high performance liquid chromatography (HPLC) can provide higher-resolution separation of materials than a preparative column. This method can be used to confirm that a purified conjugate is free of unconjugated components, or to quantify residual reactants in situations where a small percentage of a particular component may co-elute with the desired product. HPLC is also used to characterize and purify custom small molecule compounds for conjugation, as well as intermediate molecular weight conjugates such as peptide-oligonucleotide and polysaccharide conjugates.



Lanes	
1	MW markers
2	Native antibody, 3 µg
3	Conjugate Lot #1, 5 µg
2	Conjugate Lot #2, 5 µg
3	Conjugate Lot #3, 5 µg

Figure 17. Non-reducing SDS-PAGE gel results of three large-scale lots of HRP-antibody conjugate. Each lot was quenched with 2-sulfobenzaldehyde (2-SB) once the optimized 354 nm hydrazone absorbance value had been reached, stopping the reaction. Monitoring of the hydrazone absorbance during the conjugation reaction allows for unparalleled lot-to-lot reproducibility, as the conjugation reaction can be stopped by the addition of 2-SB when the desired degree of conjugation has been achieved.

Applications where a larger-scale batch may be desirable include:

- Attaching an immunogenic protein (e.g., keyhole limpet hemocyanin; KLH) to an antisense oligonucleotide, small molecule drug, peptide, or other compound to generate antibodies against that molecule.
- Attaching payloads to cell-targeting peptides, antibodies, aptamers, or small molecule ligands to guide their delivery to cells displaying the target antigen.
- Creating conjugates to be used in assays where one of the conjugation partners binds an analyte and the other component provides a means of detection, either directly (e.g., a horseradish peroxidase (HRP) conjugate) or indirectly (e.g., avidin or streptavidin conjugated to an oligonucleotide, fluor, or enzyme).

Scaling up a conjugation process with novel materials can be unpredictable, but advice from scientists with years of bioconjugation experience can help you avoid costly pitfalls. Outsourcing your project to Vector Laboratories means that your conjugate can be processed using methods and equipment optimized for large-scale conjugation, freeing up your time and minimizing the potential for loss of valuable starting materials.

For more information or to request a quote go to: **vectorlabs.com/custom-and-oem-services**.

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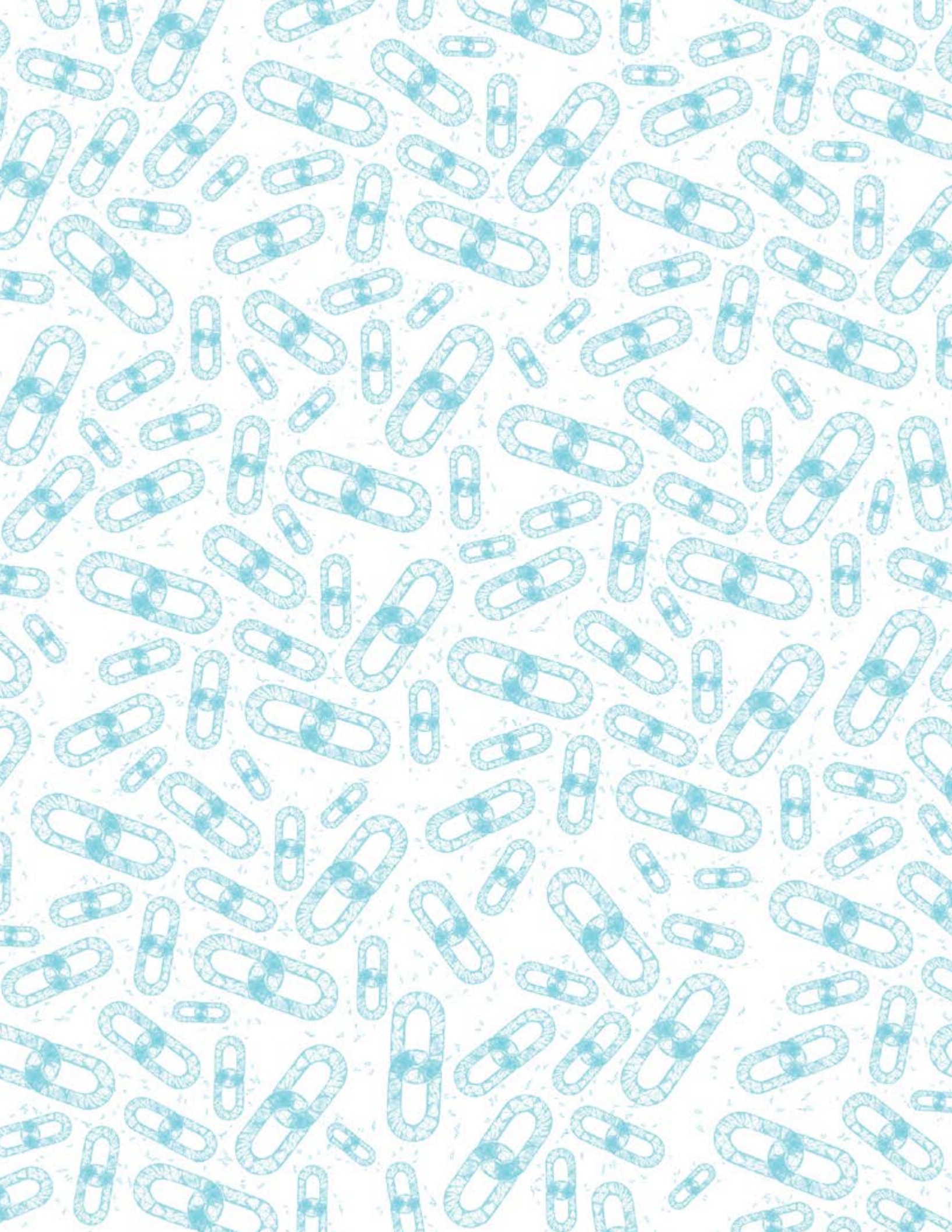

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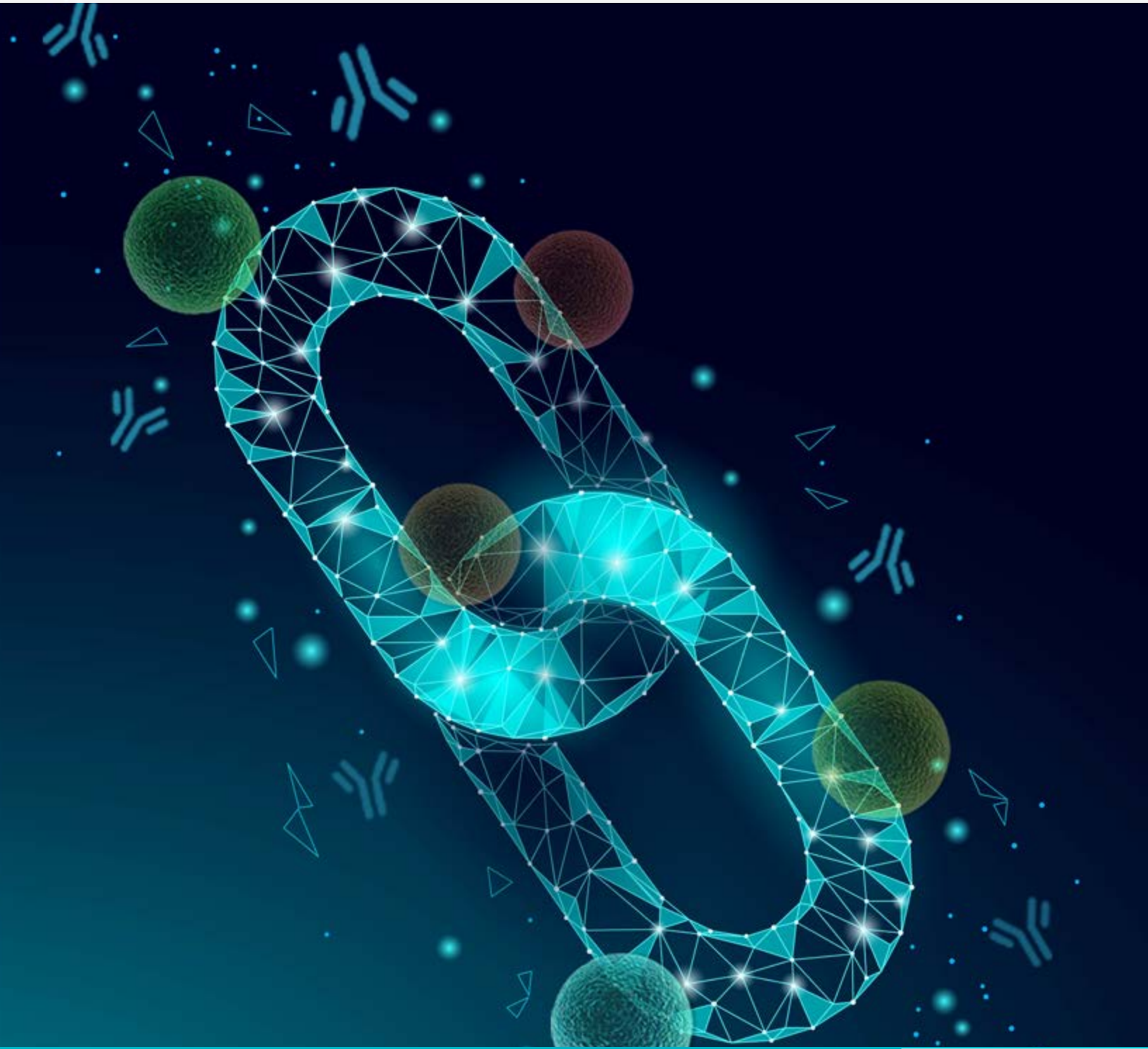

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