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# PROTEIN-OLIGONUCLEOTIDE CONJUGATION KIT

**SKU:** S-9011-1




## DESCRIPTION

The Protein-Oligo Conjugation Kit is designed to easily and efficiently prepare two separate protein-oligo conjugates. This kit is flexible so that researchers with little or no conjugation experience can make their own custom protein-oligo conjugates to suit their research needs. It includes all of the necessary components, including S-HyNic and S-4FB, for the rapid and specific crosslinking of any protein with any oligo from 20 to 100 nucleotides in length.

The molecular weight range for proteins to be conjugated with this kit is 25,000 to 900,000 Daltons. Protein amounts from 50 – 650 µg at a concentration of 1 – 5 mg/mL in a volume of 50 – 130 µL are suitable for conjugation.

## SPECIFICATIONS

| | |
|---|---|
| **Reactivity** | Amine |
| **Unit Size** | 1 Kit |

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| | |
|---|---|
| **Storage Instructions** | 2° – 8°C – Do Not Freeze |
| **Applications** | In Situ Proximity Ligation |
| **Conjugate** | Protein |
| **Label** | Oligonucleotide |

# KIT COMPONENTS

- S-HyNic (2 X 1 mg)
- S-4FB (2 x 1mg)
- 10X Modification Buffer (1.5 mL)
- 10X Conjugation Buffer (1.5 mL)
- 10X TurboLINK™ Catalyst Buffer (1.5 mL)
- 7kDa MWCO, 0.5 mL Thermo Scientific™ Zeba™ Column (4)
- Anhydrous DMF (1.5 mL)
- 2-Hydrazinopyridine (2-HP) (0.5 mL)
- 2-Sulfobenzaldehyde (2-SB) (0.5 mL)
- 7kDa MWCO 2 mL Zeba™ Column (2)
- 10X PBS (1.5 mL)
- 2 mL Collection Tube (12)
- Oligo Resuspension Solution (1.0 mL)

# TECHNICAL INFORMATION

## I. Introduction

## a. Product Description

The Protein-Oligo 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-oligo conjugate to suit their needs.

SoluLINK bioconjugation technology converts more than 95% of protein to conjugate when four mole equivalents of oligo are added. High conversion rates, coupled with the unique UV-traceable bond formed during crosslinking, allows for easy purification and identification of the conjugate from the excess oligo using size exclusion purification methods such as HPLC.

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## b. SoluLINK Bioconjugation Technology

The Protein-Oligo Conjugation Kit uses the superior SoluLINK bioconjugation technology to prepare protein-oligonucleotide conjugates in 3 easy-to-perform steps (Figure 1). The first step is the modification of the oligo with our 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 previous crosslinking methods:

1. The reaction is high yielding. Routine yields of conjugate are 50-80% based on starting protein.

2. The reaction is efficient: Only 3-4 mole equivalents of oligo are necessary for the protein, >90% of the protein is conjugated.

3. The conjugate bond is extremely stable: The conjugate bond is stable to 92℃ and pH 2.0-10.0.

4. The reaction conditions are mild and do not cause any protein denaturation: unlike thiol-based conjugation protocols, where reducing reagents are required that can compromise the activity of proteins by cleaving disulfide bonds, the HyNic-4FB conjugation couple leaves disulfide bonds intact. No metals, oxidation or reducing reagents are required.

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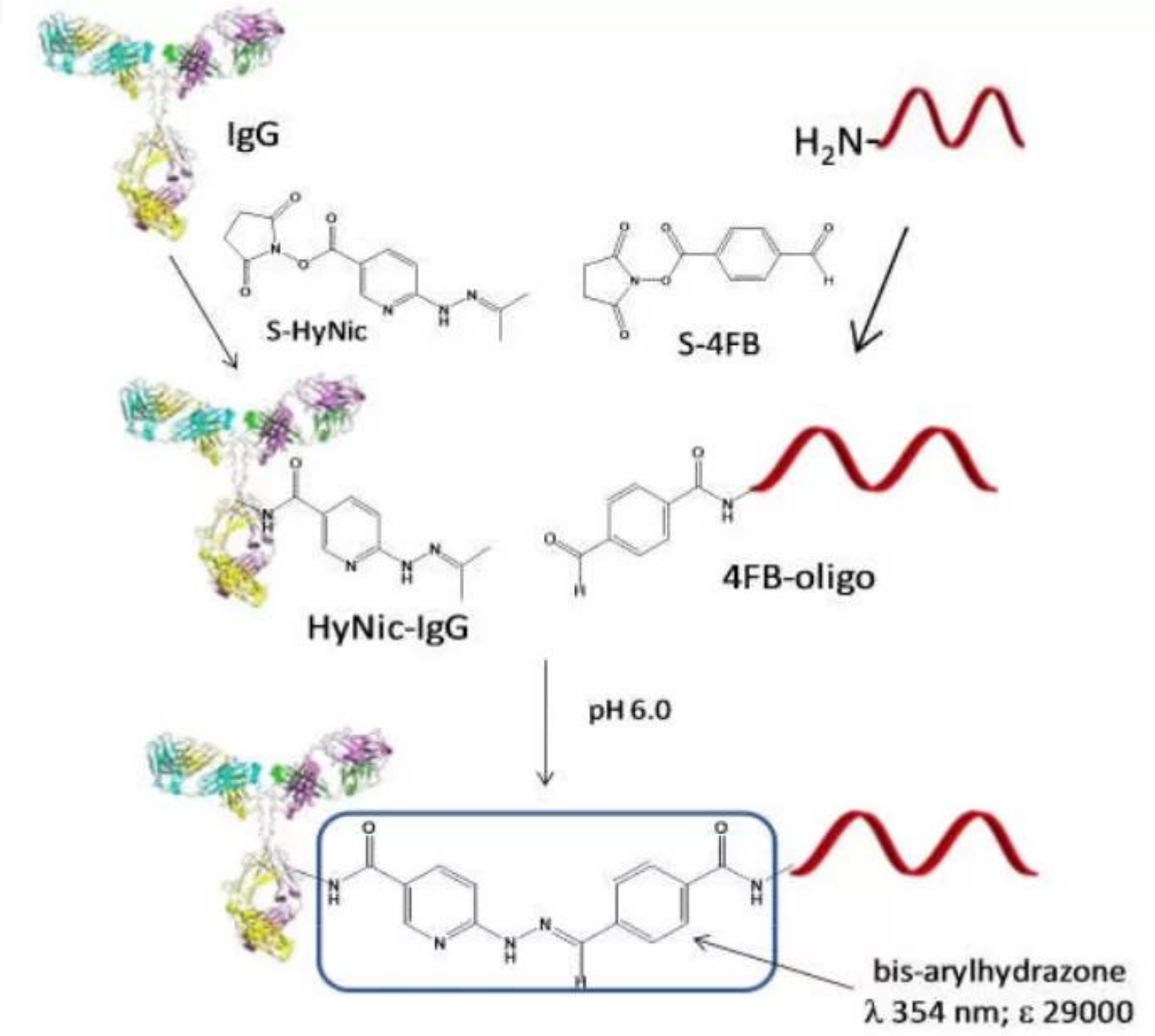



**Figure 1:** Schematic representation of the three step process to prepare an antibody-oligonucleotide conjugate using SoluLINK Bioconjugation technology. Initially an antibody is modified with S-HyNic to incorporate HyNic groups and subsequently the HyNic-modified antibody is reacted with a 4FB-modified oligonucleotide.

5. The conjugation is traceable spectrophotometrically. The HyNic-4FB conjugate bond is UV-traceable; it absorbs at 354 nm and has a molar extinction coefficient of 29,000 L/(mol*cm).

6. The modifications of both the HyNic linker on the protein and the 4FB linker on the

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oligonucleotide are quantifiable using colorimetric assays. The reproducibility of any reaction is facilitated by accurate characterization of all components. The Molar Substitution Ratio (MSR) of linker groups, i.e. the number of HyNic linkers per protein, can be quantified colorimetrically. This kit contains all the reagents necessary to determine the MSRs for both the protein and the oligo.

## II. Protein-Oligonucleotide Conjugates: A Review

The diversity and specificity of proteins combined with the specificity of hybridization of oligonucleotides results in unlimited numbers of specific protein detection reagents whose applications are addressed below.

The use of oligo-protein conjugates was initially demonstrated by Sano *et al*. for protein detection by a technique called immuno-PCR (Polymerase Chain Reaction) where a 100-mer oligo/antibody conjugate was allowed to bind to its ligand and amplified by PCR demonstrating extremely sensitive protein detection. Since this initial publication there has been a need for a straight forward, efficient and high yielding method for the preparation of these conjugates.

The first generation immuno-PCR protocol was plagued by high background due to non-specific binding of the conjugate and the extreme sensitivity of PCR. This has been overcome by the Proximal Ligation Assay (PLA) developed by Fredriksson and Lundegren. In the PLA assay, two antibodies to different epitopes are conjugated to a 40-mer 5’-phosphorylated oligonucleotide through the 3’-end and 60-mer oligonucleotide conjugated through its 5’-terminus. The two oligo/antibody conjugates are incubated with the sample, allowed to bind to their respective epitopes, the mixture is washed and then incubated with a ‘splint’ oligo that hybridizes across the two oligonucleotides that is subsequently ligated. Following ligation, PCR is performed on the ligated oligo generating a quantifiable signal. In subsequent work the oligo/antibody conjugates used by Fredriksson *et al*. and others used conjugates using the HyNic-4FB conjugation method. Kozlov *et al* also describe the use of oligonucleotide/antibody conjugates for the sensitive detection of proteins.

Additionally, oligonucleotide/antibody conjugates have been used for capture of antigens and subsequent addressing to antibody arrays for multiplex detection of proteins as well for cell sorting on the same diagnostic platform. Oligonucleotide/protein conjugates have been also been used in vaccines to increase adjuvanticity using CpG oligonucleotide/protein conjugates.

## III. Accessing 4FB-Modified Oligonucleotides

Stable and disulfide-cleavable 4FB oligonucleotides can be obtained in several ways:

1. **5’-4FB oligonucleotide**

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***a.* 4FB-phosphoramidite**: 4FB-Phosphoramidite (1; Figure 2) is available for incorporation of 5’-4FB groups during oligonucleotide solid phase synthesis. Standard coupling protocols are used and the yields are similar to any amino modifier.

***b.* 5’-amino oligonucleotides:** 5’-amino oligonucleotides may be converted to 5’-4FB modified oligonucleotides in a straightforward high yielding modification step with S-4FB (2; Figure 2).

The Protein-Oligo Conjugation Kit includes S-4FB and all the reagents and materials required to convert a 5’-amino oligonucleotide to a 5’-4FB-oligonucleotide.

2. **3’-4FB oligonucleotide:**
   3’-Amino oligonucleotides are converted to 3’-4FB modified oligonucleotides in an easy, high yielding modification step with S-4FB (2; Figure 2).
   The Protein-Oligo Conjugation Kit includes S-4FB and all the reagents and disposables required to convert a 3’-amino oligonucleotide to a 3’-4FB-oligonucleotide.
3. **5’- and 3’-4FB disulfide-cleavable oligonucleotides:** 5’- and 3’-amino oligonucleotides may be converted to disulfide-cleavable oligonucleotides using S-SS-4FB (3; Figure 2) in an easy, high yielding modification step.

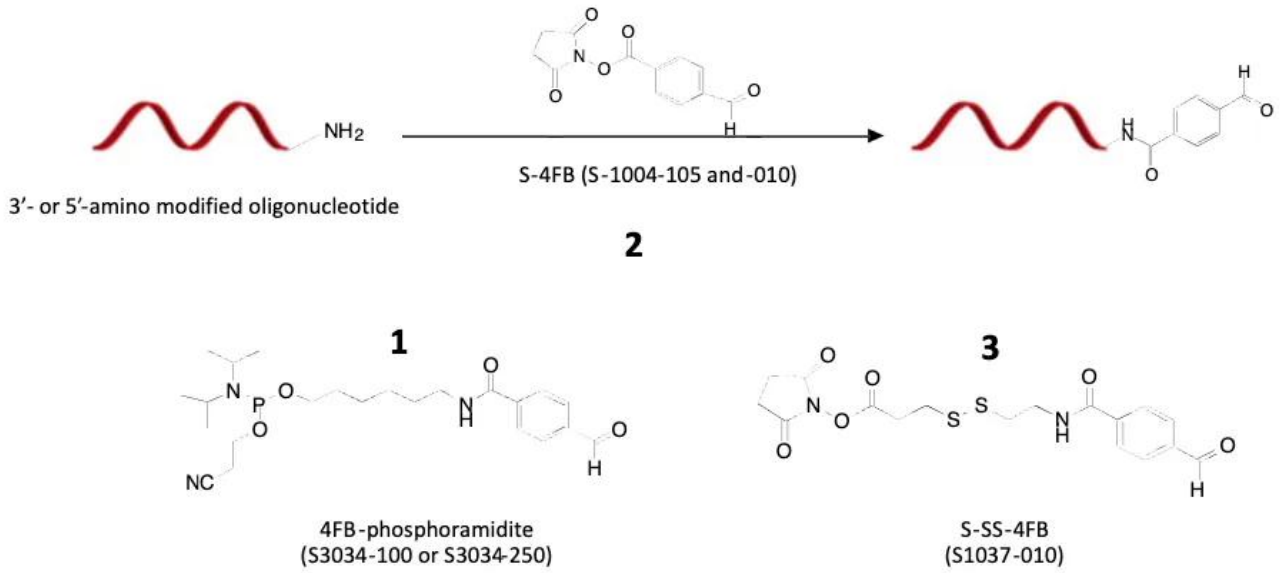



**Figure 2:** Schematic representation of the conversion of an amino-modified oligonucleotide to a 4FB-oligonucleotide with S-4FB (2) (top) and structures of 4FB-phosphoramidite (1) and S-SS-4FB (3), the reagent used to convert an amino-oligonucleotide to a 4FB-SS-oligonucleotide.

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### IV. The Keys to Successful Conjugation

The following are three crucial requirements that must be fulfilled for a reproducibly successful preparation of a protein-oligonucleotide conjugate using the SoluLINK bioconjugation technology:

1. **Desalting:** Prior to modification, the starting protein must be thoroughly desalted, removing all amine contaminants, and exchanged into 1X Modification Buffer.
2. **Protein concentration:** The recommended concentration of the protein (1–5mg/ml) must be adhered to in all steps.
3. **Molar substitution ratio:** The molar ratio of the HyNic on the protein and the 4FB on the oligo must be determined and within the desired range before continuing to the next step.

# CITATIONS

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# DOCUMENTS

- User Guide
- Bioconjugation White Paper
- Safety Data Sheet

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- S-9011 – Protein-Oligonucleotide Conjugation Calculator
- Troubleshooting Guide – Bioconjugation
- BCA Protein Assay Protocol
- Bradford Assay Protocol
- Download CoA
- Datasheet

# GALLERY IMAGES



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