Antibody-Oligonucleotide Conjugate Preparation and Applications

Introduction: *Antibody-oligonucleotide conjugates have the potential to be the platform tool to perform more sensitive and multiplexed detection protein assays. Combining the diversity and specificity of the binding of antibodies to their antigen with the diversity and specificity of hybridization of oligonucleotides into an antibody-oligonucleotide conjugate results in the ability to produce unlimited numbers of more sensitive protein specific detection reagents. Life science and diagnostic uses of antibody-oligo conjugates that improve the detection of biomarkers have included immunoPCR[1], PLA (Proximity Ligation Assay)[2], and ECPA (Electrochemical Proximity Assay)[3] to name a few.*

To realize the far reaching potential of antibody-oligonucleotide conjugates methods and kits to prepare multiple antibody-oligonucleotide conjugates using affordable quantities of antibodies, would ideally be 100 µg, without the requirement for purification by chromatography. These are not insignificant criteria to satisfy. Since Sano et al. [4] published their results employing antibody-oligonucleotide conjugates for the detection of proteins using PCR in a technique called immunoPCR, there has been straightforward, efficient and high yielding chemistry developed for the preparation of these conjugates.

A second generation more sensitive iPCR assay with significantly lower background called the Proximal Ligation Assay (PLA) has been developed by Fredriksson et al. [2] In the PLA assay two antibody-oligonucleotide conjugates against the same target but different epitopes are allowed to bind

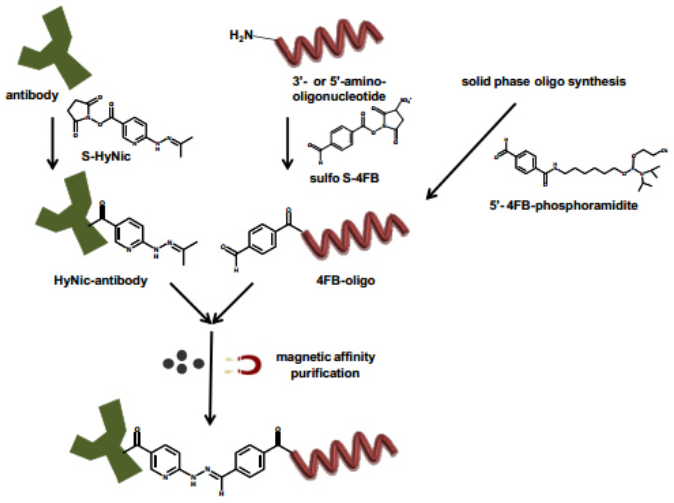

Figure 1. Schematic representation of the two step process to prepare an antibody-oligonucleotide conjugate using Solulink's bioconjugation chemistry. Initially a 3'- or 5'-amino-modified oligonucleotides oligonucleotide is 4FB-modified with Sulfo-S-4FB or by solid phase oligonucleotide synthesis using 4FB-phosphoramidite (1), followed by modification of the antibody with S-HyNic to incorporate HyNic groups. The HyNic-modified antibody is then reacted with 4FB-modified oligonucleotide to yield a bis-arylhydrazone mediated conjugate.

followed by the addition of a 'splint' oligo that hybridizes across the two oligos followed sequentially by a ligation reaction and PCR. 5.6. Fredriksson has subsequently shown that the PLA assay can be engineered to simultaneously detect multiple proteins in a single sample.

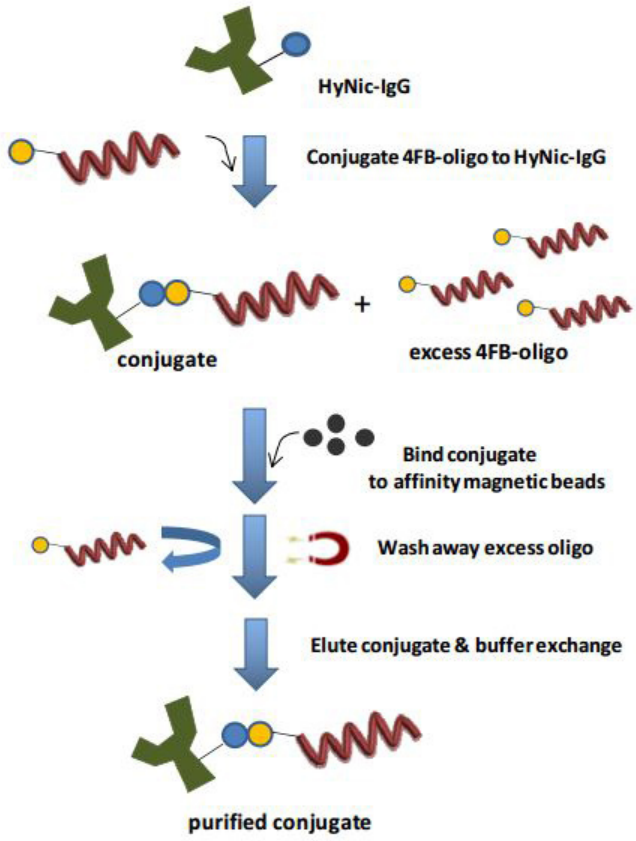

Figure 2. Step 1 HyNic-modified antibody is conjugated to 3'- or 5'-4FB oligonucleotide converting >95% of antibody to oligonucleotide conjugate. Step 2: The conjugate is adsorbed onto affinity magnetic beads and the non-adsorbed excess oligonucleotide is removed by simple magnetization and removal of supernatant. Step 3: The purified conjugate is isolated by desorption from the magnetic beads with elution buffer followed by exchange into storage buffer. The overall yield is 30-50% based on starting antibody.

Heath et al.[7, 8] have demonstrated the use of antibody-oligonucleotide conjugates for multiplexed protein detection using microfluidic based arrays. Kozlov et al.[10] have also reported the use of antibody-oligonucleotide conjugates for sensitive detection of proteins.

Preparing antibody-oligonucleotide conjugates without chromatography:

Solulink offers the "All-in-One Antibody-Oligonucleotide Conjugation Kit", which has been referenced and used by researchers for several years, provides scientists with an all-inclusive kit that produces antibody-oligonucleotide conjugates starting with 100 µg of antibody in high yield and purity without the need for chromatographic purification. This technology permits the simultaneous preparation of multiple conjugates on a bench top requiring only pipettes, a microcentrifuge and a UV spectrophotometer.

The antibody-oligonucleotide product is **>95%** free from unconjugated antibody and oligonucleotide using only a small excess of oligonucleotide.

Two breakthroughs make this long awaited technology possible.

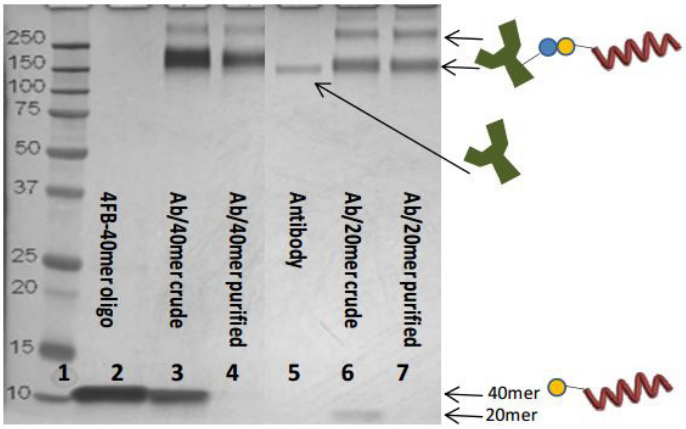

Figure 3. The silver stained SDS-PAGE presents data for the conjugation and purification of a 40-mer (Lanes 2 and 3) and a 20-mer (Lanes 6 and 7) 4FB-oligonucleotides to HyNic-modified antibodies. In the case of the 40-mer oligonucleotide/ antibody conjugate it is clearly evident that there is virtually no free antibody in the conjugate. In both purified conjugates there is no visible free oligonucleotide. The 'thick' conjugate bands are due to a distribution of 2-4 oligonucleotides conjugated to each antibody.

First, Solulink's HyNic/4FB bioconjugation linkage system as applied to the conjugation of oligonucleotides with antibodies is stoichiometrically efficient and high yielding converting >95% antibody to antibody-oligonucleotide conjugate (Figure 1). Furthermore, conjugations of oligomers of 20-60 nucleotides are conjugated with equal efficiency. The method is extremely mild as no metals, reductants or oxidants are used in the conjugation step. Further enhancing the efficiency of conjugation is the use of aniline as a reaction catalyst (Dirksen et al. 11, 12, 13) In a standard conjugation protocol 5 equivalents of 4FB-oligonucleoitde is used resulting in the conjugation of 2-3 oligonucleotides per antibody. A 3'- or 5'- 4FB-modified oligonucleotide can be prepared by modification of an amino-modified oligonucleotide with Sulfo-S-4FB or a 5'-4FB-oligonucleotide can be synthesized directly during the solid phase synthesis of the oligonucleotide using a 4FB-phosphoramidite available from Solulink.

The second breakthrough was the application of a method to isolate the conjugate by conjugate adsorption to a proprietary magnetic affinity matrix that allows removal of excess 4FB-oligonucleotide followed by elution of the purified conjugate using mild elution buffers (Figure 2). The overall yield of the antibody-oligonucleotide conjugate is 30-50% based on antibody recovery. The conjugate is >95% free from unconjugated HyNic-antibody and 4FB-oligonucleotide. Multiple conjugates can be prepared simultaneously satisfying the requirement for the use of this protocol to prepare antibody-oligonucleotide conjugates for highly multiplex detection of antigens. The bis-arylhydrazone conjugate bond is stable to both heat (94oC) and pH (3 and 10). Figure 3 presents typical conjugation results as visualized on an SDS-PAGE gel. Both a 20-mer and a 60-mer are conjugated to an antibody using the Antibody-Oligonucleotide All-in-One Conjugation Kit. As is readily apparent in the gel, very little un-conjugated antibody or un-conjugated oligonucleotide is present in the purified conjugate.

Summary:

Preparation of antibody-oligonucleotide conjugates using Solulink's Antibody-Oligonucleotide All-in-One Conjugation Kit allows scientists to produce multiple antibody-oligonucleotide conjugates on their benchtop without the need for chromatographic purification.

References:

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Product information:

Cat. No. A-9202-001

Antibody-Oligonucleotide All-in-One Antibody Conjugation Kit—Conjugates 100 µg of antibody

Manuals and Protocols

Calculator

Certificate of Analysis

MSDS

Frequently Asked Questions

Is there a limit to the size of my oligo?

Yes. The kit is designed to conjugate oligos between 20 and 60 bases though some users have used oligos up to 120 bases. For oligos outside of this size range, please contact our technical support team for advice.

Does my oligo have to be HPLC purified?

Yes. The oligo MUST be HPLC purified due to oligo synthesis reactants interfering with the conjugation chemistry.

Does Solulink offer a custom oligo conjugation service?

Yes. Solulink does offer a custom oligo conjugation service. Please contact our technical support team for more information or call +1.858.625.0670.

Do I need specific functional groups on my oligo?

Yes. The oligo must contain a terminal amine or 4FB group. This amine group is added during oligo synthesis and may be either 5′ or 3′. The 4FB can be added during oligo synthesis by our partner TriLink Biotechnologies.

Can I use the kit to conjugate oligos to proteins other than antibodies?

No, Solulink's Antibody-Oligonucleotide Conjugation Kit is primarily designed to conjugate oligos to purified antibodies. However, Solulink does have a Protein-Oligo Kit that does two conjugations (S-9011-1). Please contact our technical support team for more information.

Does antibody species or isotype make a difference to the conjugation efficiency?

No. The conjugation system is primarily designed to conjugate antibodies to purified IgG. The kit will conjugate oligos to IgG irrespective of species. The kit will also conjugate all other antibody sub-types. Please ccontact our technical support team for specific advice.

Can I use the Solulink Antibody-Oligonucleotide Conjugation Kit to conjugate double-stranded DNA or RNA to antibodies?

Yes. It works best with DNA or RNA of <60bp. For advice and support on conjugating other forms of nucleic acids please contact our technical support team.

Are larger pack sizes available?

Yes. The standard kit is designed to conjugate 100 µg of antibody. Solulink can provide larger kits upon request. Please contact our sales team for more information.