WHITE PAPER

Antibody-Oligonucleotide Conjugate Preparation

vector LABORATORIES
Together we breakthrough™

Introduction

The far-reaching potential of antibody-oligonucleotide conjugates has yet to be fully realized. Methods have not been developed to prepare multiple antibody-oligonucleotide conjugates using affordable quantities of antibodies (e.g., 100 µg), without the requirement for purification by chromatography. Previously, it has been difficult to overcome the challenges needed to satisfy these criteria.

Seeing the potential as a platform tool for diagnostics.

Highly multiplexed protein diagnostic assays based on antibody-oligonucleotide conjugates could become a popular platform of choice. 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 high numbers of protein-specific detection reagents.

Since Sano *et al.*[1] published their results employing antibody-oligonucleotide conjugates for the detection of proteins using PCR in a technique called immuno-PCR there has been a need for a straightforward, efficient and high yielding chemistry for the preparation of these conjugates.

An improvement upon significantly lower background named the Proximity 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 and is followed by the addition of a 'splint' oligo that hybridizes across the two oligos followed sequentially by a ligation reaction and PCR.[3, 4]

Fredriksson[5] has subsequently shown that the PLA assay can be engineered to simultaneously detect multiple proteins in a single sample.

Heath *et al.*[6, 7] have demonstrated the use of antibody-oligonucleotide conjugates for multiplexed protein detection using microfluidic based arrays. Kozlov *et al.*[8] have also reported the use of antibody-oligonucleotide conjugates for sensitive detection of proteins.

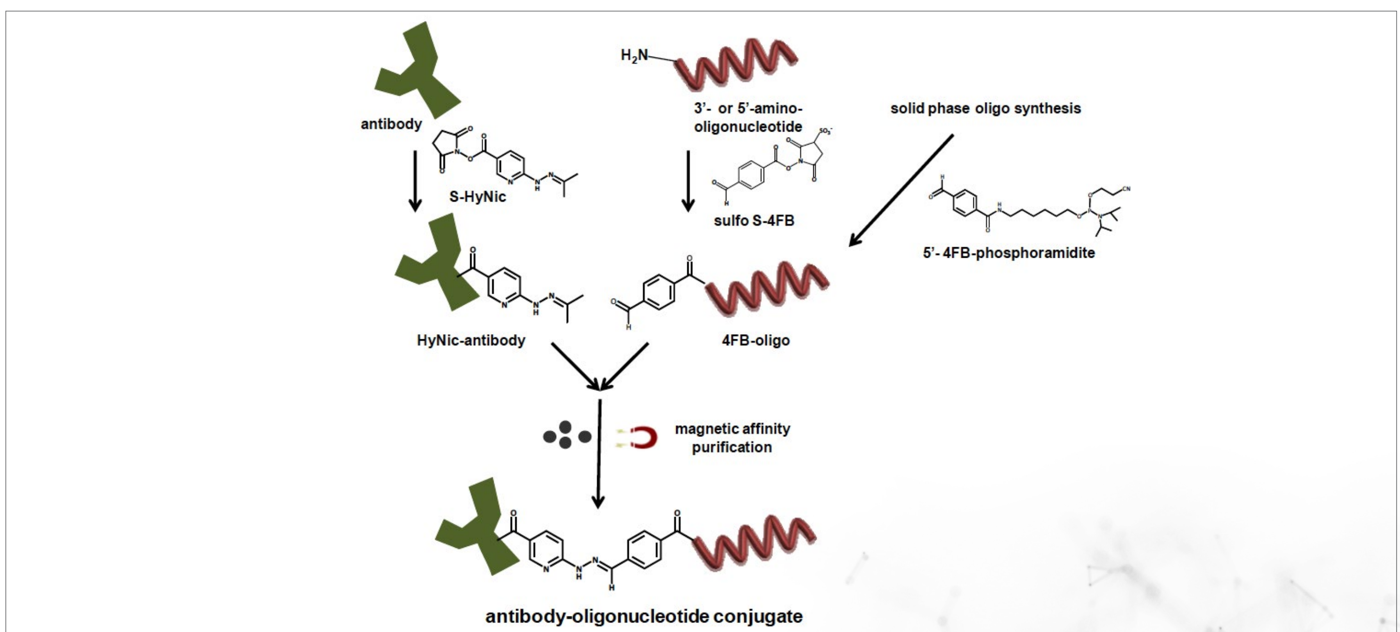

Figure 1 *Schematic representation of the two step process to prepare an antibody-oligonucleotide conjugate using SoluLINK® bioconjugation technology. Initially a 3'- or 5'-amino-modified oligonucleotide is 4FB-modified with Sulfo-S-4FB or by solid phase oligonucleotide synthesis using 4FB-phosphoramidite (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.*

Preparing antibody-oligonucleotide conjugates without chromatography:

The Antibody-Oligonucleotide All-in-One™ Conjugation Kit 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 benchtop requiring only micropipettes, 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.

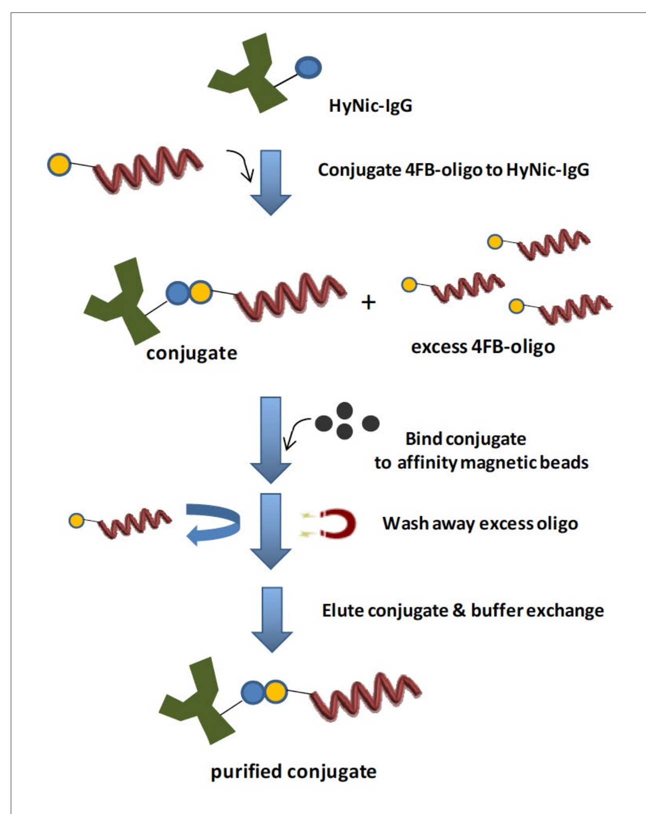

Figure 2 *Step 1: HyNic-modified antibody is conjugated to 3'- or 5'-4FB oligonucleotide converting >95% of antibody to oligonucleotide conjugate. Step 2: The conjugate is adsorbed onto affinity magnetic beads and the non-adsorbed excess oligonucleotide is removed by simple magnetization and removal 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.*

Applying breakthrough technology.

The first technology breakthrough was the HyNic-4FB bioconjugation 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.*[9, 10, 11]) In a standard conjugation protocol 5 equivalents of 4FB-oligonucleoitide 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.

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 (94°C) and pH (pH 3-10). Figure 3 presents typical conjugation results as visualized on an SDS-PAGE gel. Both a 20-mer and a 40-mer are conjugated to an antibody using the Antibody-Oligonucleotide All-in-One Conjugation Kit. As is readily apparent in the gel, very little unconjugated antibody or unconjugated oligonucleotide is present in the purified conjugate.

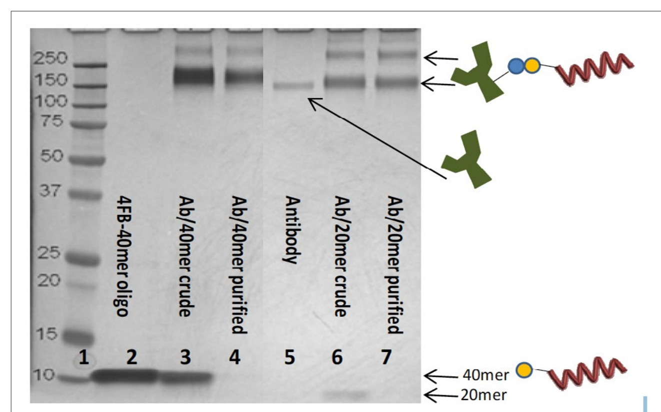

Figure 3 *Silver stained SDS-PAGE presents data for the conjugation and purification of a 40-mer (Lanes 2 and 3) and a 20-mer (Lanes 6 and 7) 4FB-oligonucleotides to HyNic-modified antibodies. In the case of the 40-mer oligo-antibody conjugate, it is evident that there is virtually no free antibody in 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. The overall yield is 30–50% based on starting antibody.*

Summary

Preparation of antibody-oligonucleotide conjugates using the Antibody-Oligonucleotide All-in-One Conjugation Kit allows production of multiple antibody-oligonucleotide conjugates on the benchtop without the need for chromatographic purification.

Published References

1. Sano T, et al. 1992. Immuno-PCR: Very Sensitive Antigen Detection by Means of Specific Antibody-DNA Conjugates. *Science.*
2. Fredriksson S, et al. 2002. Protein detection using proximitydependent DNA ligation assays. *Nature Biotechnology.*
3. Fredriksson S, et al. 2008. Multiplexed Proximity Ligation Assays to Profile Putative Plasma Biomarkers Relevant to Pancreatic and Ovarian Cancer. *Clinical Chemistry.*
4. Fredriksson S, et al. 2007. Multiplexed protein detection by proximity ligation for cancer biomarker validation. *Nature Methods.*
5. Bailey RC, et al. 2007. DNA-Encoded Antibody Libraries: A Unified Platform for Multiplexed Cell Sorting and Detection of Genes and Proteins. *J. Amer. Chem. Soc.*
6. Fan R, et al. 2007. Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood. *Nature Biotechnology.*
7. Kwong GA, et al. 2009. Modular nucleic acid assembled p/MHC microarrays for multiplexed sorting of antigen-specific T cells. *J. Amer. Chem. Soc.*
8. Kozlov IA, et al. 2004. Efficient strategies for the conjugation of oligonucleotides to antibodies enabling highly sensitive protein detection. *Biopolymers.*
9. Dirksen A, et al. 2006. Nucleophilic Catalysis of Hydrazone Formation and Transimination: Implications for Dynamic Covalent Chemistry. *J Am Chem Soc.*
10. Dirksen A, et al. 2006. Nucleophilic Catalysis of Oxime Ligation, Angew. *Chem. Int. Ed.*
11. Dirksen A, et al. 2008. Rapid Oxime and Hydrazone Ligations with Aromatic Aldehydes for Biomolecular Labeling. *Bioconjugate Chem.*

For custom bioconjugation services visit

vectorlabs.com/custom-and-oem-services

For more information please visit

vectorlabs.com/solulink

©2022 Vector Laboratories. All rights reserved.
For research use only. Not intended for animal or human therapeutic or diagnostic use.
All-in-One and SoluLINK are trademarks of Vector Laboratories, Inc.

LIT3029. Rev.01