# Antibody-Oligonucleotide Conjugate Preparation

The far reaching potential of antibody-oligonucleotide conjugates has yet to be fully realized as methods have not been developed to prepare multiple antibody-oligonucleotide conjugates using affordable quantities of antibodies, *i.e.* 100 μg, without the requirement for purification by chromatography. Until recently, these are not insignificant criteria to satisfy.

**Antibody-oligonucleotide conjugates have the potential to be the platform tool to perform highly multiplexed protein diagnostic 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 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 immunoPCR there has been a need for a straightforward, efficient and high yielding chemistry for the preparation of these conjugates.

A second generation more sensitive iPCR assay with significantly lower background named 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

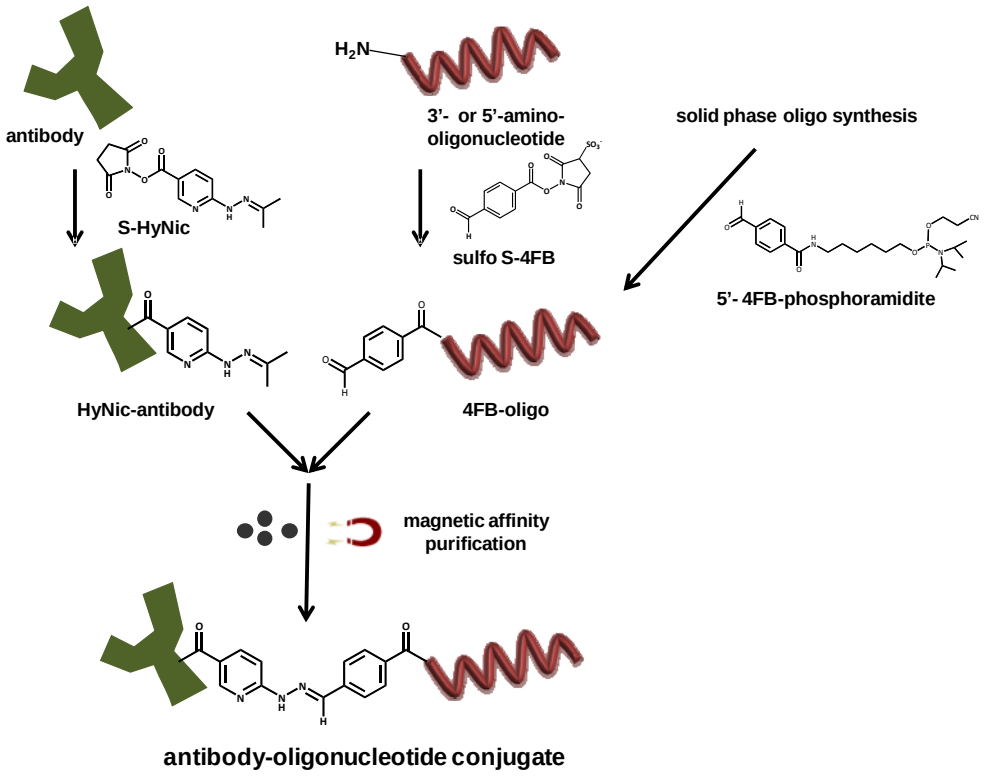


**Figure 1:** Schematic representation of the two step process to prepare an antibody-oligonucleotide conjugate using Solulink's bioconjugation chemistry. Initially a 3'- or 5'-amino-modified oligonucleotides oligonucleotide is 4FB-modified with Sulfo-S-4FB or by solid phase oligonucleotide synthesis 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.

bind 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.

**Preparing antibody-oligonucleotide conjugates without chromatography:**

Solulink now offers the "All-in-One Antibody-

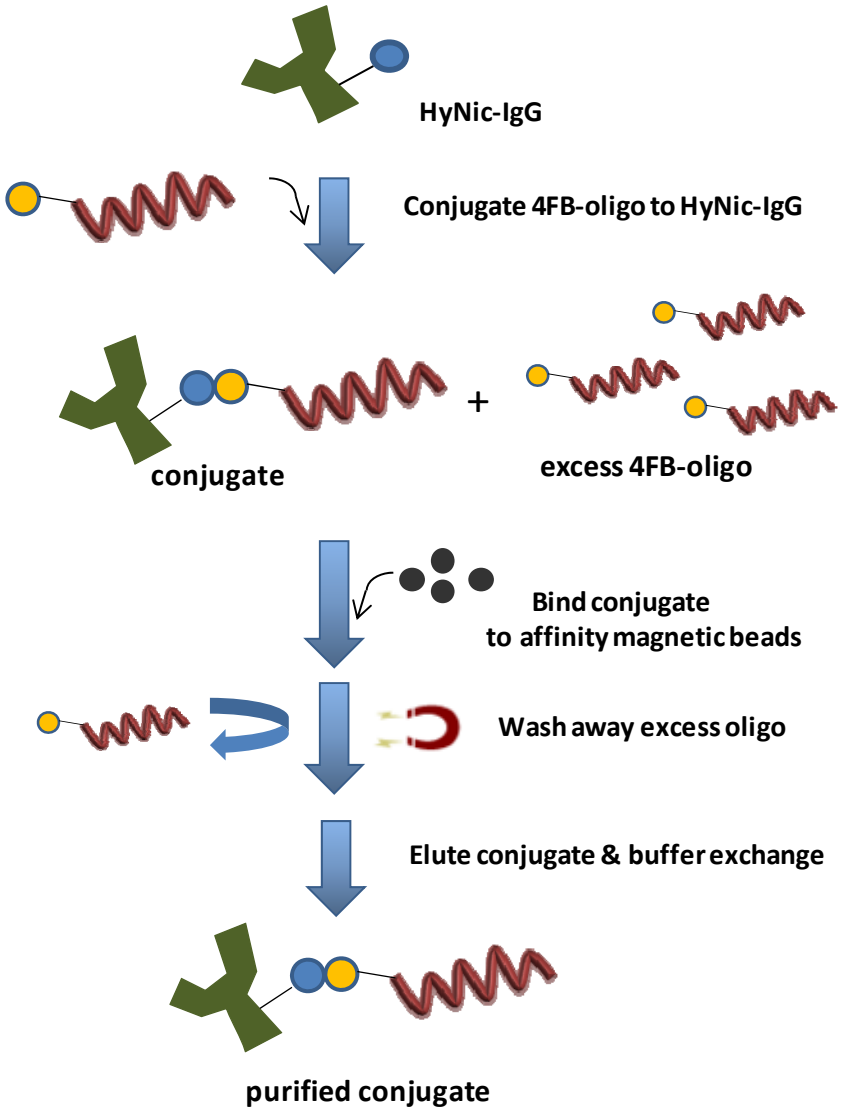


**Figure 2:** Step 1 HyNic-modified antibody is conjugated to 3'- or 5'-4FB oligonucleotide converting >95% of antibody to oligonucleotide conjugate. Step 2: The conjugate is adsorbed onto affinity magnetic beads and the non-adsorbed excess oligonucleotide is removed by simple magnetization and removal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

Oligonucleotide Conjugation Kit" that 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 pipetmen, 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.**

First, Solulink's HyNic-4FB bioconjugation couple 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-oligonucleoitde is used resulting in the conjugation of 2-3 oligonucleotides-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-

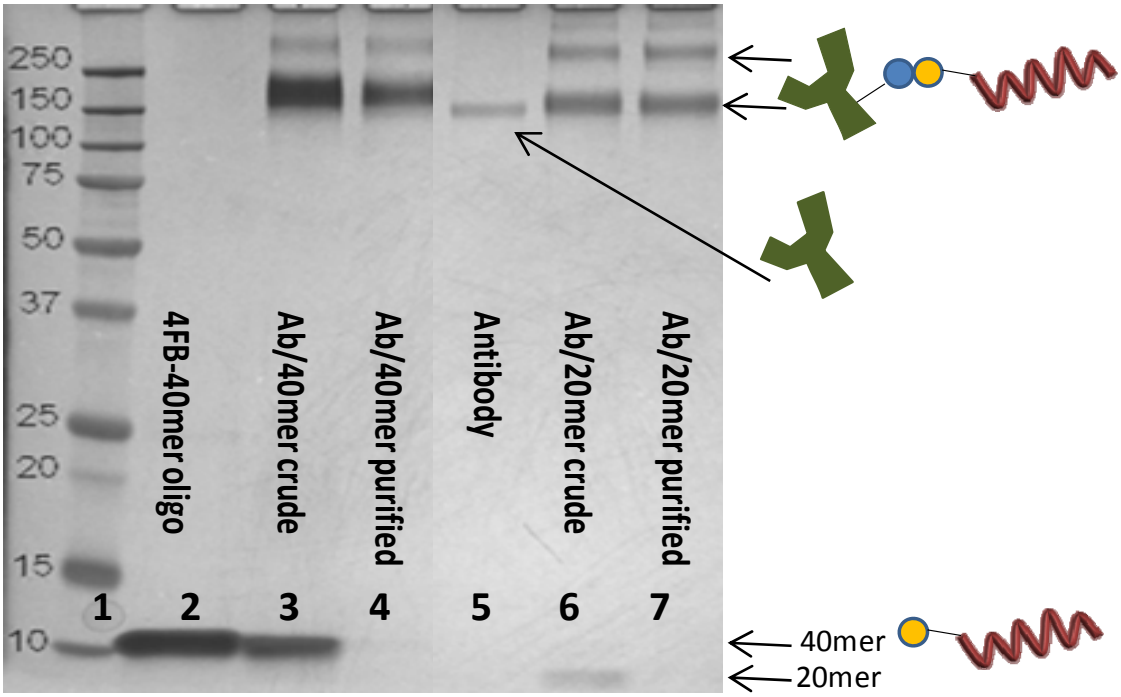


**Figure 3:** The silver stained SDS-PAGE presents data for the conjugation and purification of a 40-mer (Lanes 2 and 3) and a 20-mer (Lanes 6 and 7) 4FB-oligonucleotides to HyNic-modified antibodies. In the case of the 40-mer oligonucleotide-antibody conjugate it is clearly evident that there is virtually 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.

oligonucleoitde 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$^{o}$C) 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 All-in-One Antibody-Oligonucleotide 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 All-in-One Antibody-Oligonucleotide Conjugation Kit allows scientists to produce multiple antibody-oligonucleotide conjugates on their benchtop without the need for chromatographic purification.

**References:**

1) Sano, T., C.L. Smith, and C.R. Cantor, Immuno-PCR: very sensitive antigen detection by means of specific antibody-DNA conjugates. Science, **1992**. *258*, 120-2.

2) S. Fredriksson, M. Gullberg, J. Jarvius1, C. Olsson, K. Pietras, S.M Gústafsdóttir, A. Östman and U. Landegren, Protein detection using proximity-dependent DNA ligation assays, Nature Biotechnology **2002**, *20*, 473 – 477.

3) Fredriksson, S., Horecka, J., Brustugun, O.T., Schlingemann, J., Koong, A., Tibshirani, R. and Davis, R., Multiplexed Proximity Ligation Assays to Profile Putative Plasma Biomarkers Relevant to Pancreatic and Ovarian Cancer, Clinical Chemistry, **2008**, *54*, 582-589.

4) Fredriksson, S., Dixon, W., Ji, H., Koong, A., Mindrinos, M. and Davis, R., Multiplexed protein detection by proximity ligation for cancer biomarker validation , Nature Methods **2007**, *4*, 327.

5) Bailey, R.C., Kwong, G.A., Radu, C.G., Witte, O.W. and Heath, J.R., DNA-Encoded Antibody Libraries: A Unified Platform for Multiplexed Cell Sorting and Detection of Genes and Proteins, J. Amer. Chem. Soc., **2007**, *129*, 1959-1967.

6) R. Fan, O. Vermesh, A. Srivastava, B. K. H. Yen, L. Qin , H. Ahmad , G. A. Kwong , C.-C. Liu , J. Gould , L. Hood and J. R. Heath, Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood, Nature Biotechnology **2008,** 26, 1373 - 1378

7) G.A. Kwong, C.G. Radu, K. Hwang, C.J. Shu, C. Ma, R.C. Koya, B. Comin-Anduix, S.R. Hadrup, R.C. Bailey, O. Witte, O., N. Ton, N. Schumacher, Antoni Ribas and James R. Heath, Modular nucleic acid assembled p/MHC microarrays for multiplexed sorting of antigen-specific T cells, J. Amer. Chem. Soc. **2009**, *131*, 9695.

8) Kozlov, I.A., Melnyk, P.C., Stromsborg, K.E., Chee, M.S., Barker, D.L., Zhao, C., Efficient strategies for the conjugation of oligonucleotides to antibodies enabling highly sensitive protein detection, Biopolymers **2004**, *73*, 621.

9) Dirksen, A., Dirksen, S., Hackeng, T.M. and Dawson, P.E., Nucleophilic Catalysis of Hydrazone Formation and Transimination: Implications for Dynamic Covalent Chemistry, J Am Chem Soc, **2006**, *128*, 15602-3.

10) Dirksen, A., Hackeng, T.M. and Dawson, P.E., Nucleophilic Catalysis of Oxime Ligation, Angew. Chem. Int. Ed., **2006**, *45*, 7581 –7584.

11) Dirksen, A. and Dawson, P.E., Rapid Oxime and Hydrazone Ligations with Aromatic Aldehydes for Biomolecular Labeling, Bioconjugate Chem., **2008**, *19*, 2543-2548.