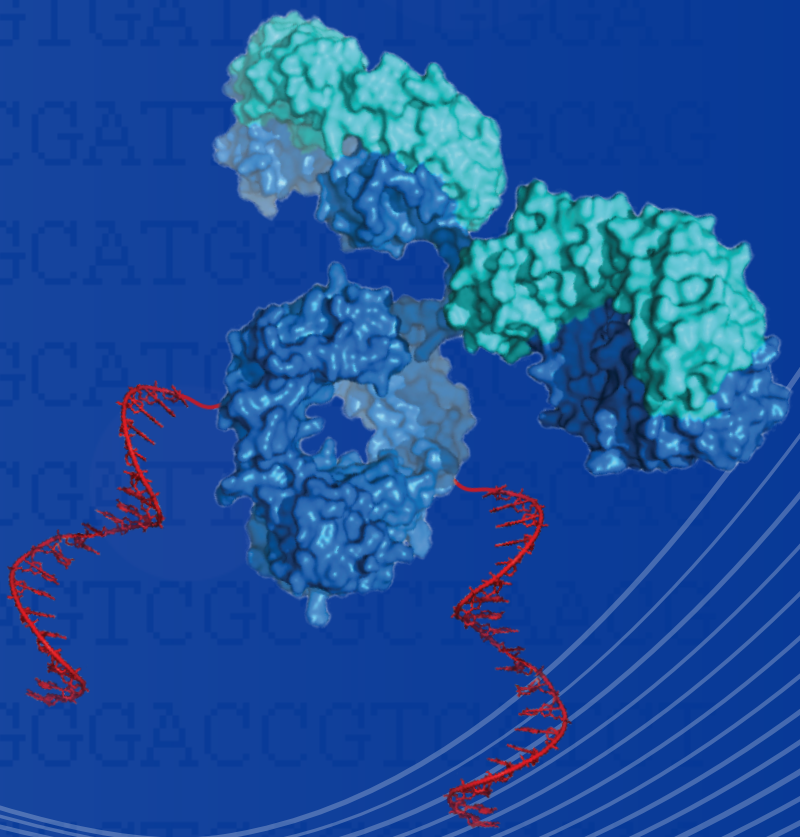
alpha Thera

eBook

Antibody-Oligo Conjugation:

Key Applications, Challenges & The Next-Gen Technology That Simplifies Labeling

February 2022



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Introduction

Antibody-oligonucleotide conjugates are becoming increasingly popular across the life sciences for their use in the precise detection of very small quantities of proteins.
In particular, there is rapidly growing interest in their use for research at the single-cell level, with applications such as CITE-seq using antibody-oligo conjugates to identify new cell types and cell states associated with disease.

Furthermore researchers are recognizing the benefits of using antibody-oligo conjugates to significantly improve assay sensitivity and specificity, with a rise in popularity for applications such as immuno-PCR and Proximity Ligation Assay (PLA).

The benefits of using of antibody-oligo conjugates within research are clear and extend from therapeutics to diagnostics. However the actual process of conjugation is still a barrier for many researchers as antibody-oligo labeling is notorious for being expensive, complex and requiring high levels of expertise. In this eBook we take a look at:

- The key applications using antibody-oligo conjugates
- The challenges and limitations of oligo conjugation
- New technologies enabling easy antibody-oligo labeling

1 The key applications using antibody-oligo conjugates

CITE-seq

CITE-seq (Cellular Indexing of Transcriptomes and Epitopes by Sequencing) developed by the New York Genome Center (NYGC), is an innovative technique for studying single-cell biology, enabling researchers to analyze individual cells, distinguish between different cell types, and study mechanisms of disease at the cell level.

CITE-seq uses antibody-oligonucleotide conjugates to measure specific cell surface proteins while also sequencing the mRNA of the single cells, providing a simultaneous view of the proteome and transcriptome. The resulting extensive data sets provide an exciting opportunity in the study of biological mechanisms of disease.

The CITE-seq process

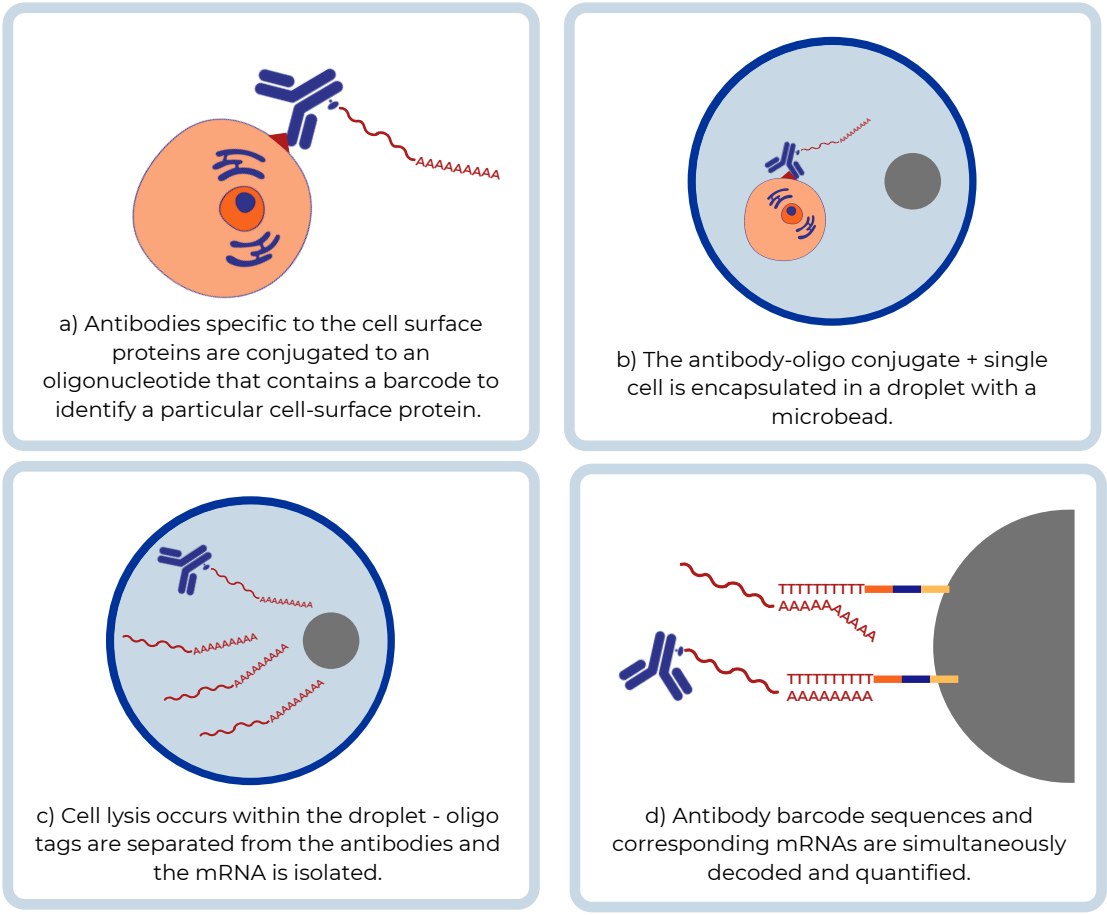


Figure 1. The CITE-seq proces

One of the most crucial elements of CITE-seq is the design of the antibody-oligonucleotide conjugates. It is imperative that there is no variability in the number of DNA tags on the antibodies, or disruption to the antibody binding site, which can be difficult to achieve with many commercially available antibody-oligo conjugation kits because of their amine-directed technology.

Analyzing the protein journey within the nucleus

CITE-seq has since developed further to be used to assess the protein journey within the nucleus. The development of inCITE-seq was led by Hattie Chung, postdoctoral associate at the Broad Institute of MIT and Harvard.[1] Check out this recent article in Nature Methods - 'How single-cell multi-omics builds relationships' - featuring an interview with AlphaThera, that takes a looks at approaches to analyze single-cell changes, and reviews inCITE-seq as a way to multiplex measurements of a protein's journey in the nucleus. The challenges of attaching DNA tags to track antibodies in the nucleus, and the requirements for cheaper and controlled ways for conjugating DNA to antibodies are also discussed. In the interview, AlphaThera members discuss the development and commercialization of oYo-Link antibody labeling technology, and the opportunities it provides researchers within the field of multi-omic single-cell analysis. Read the article here.

Immuno-PCR: A powerful method of immunodetection

Immuno-PCR is a method of immunodetection combining the specificity of an ELISA with the signal amplification of PCR. Enabled by antibody-oligonucleotide conjugates, iPCR detection assays have shown to be 100-1000 fold more sensitive than traditional ELISA and require much less antibody. An ELISA uses conjugated antibodies to detect any protein, meaning it can be highly specific; however it may not be sensitive enough to detect very low levels of protein. RT-PCR cannot be used directly for antigen detection as it does not use antibodies, however it provides exponential signal amplification.

By combining these two assay formats using an antibody-oligonucleotide conjugate, immuno-PCR provides the best of both worlds. Furthermore, it is possible to convert any type of ELISA to an immuno-PCR assay by replacing the detection antibody with an antibody-oligo conjugate.

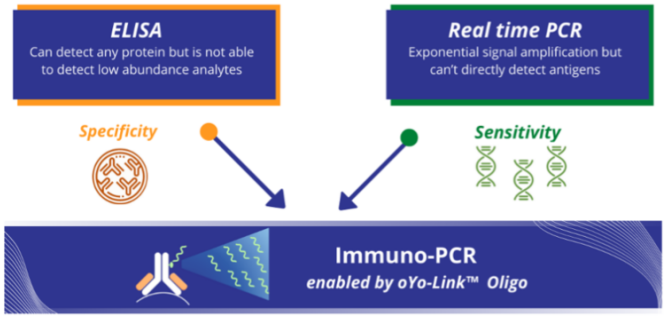


Figure 2. Immuno-PCR combines the specificity of an ELISA with the sensitivity of PCR

Performing an immuno-PCR assay

An antibody specific to the target antigen is immobilized on the surface of a microtiter plate. The washing removes any unbound antibody. The plate is then blocked and the sample added, followed by further washing to remove any unbound sample. Then a second specific antibody, coupled to an oligonucleotide is added, followed by more washing steps, before the DNA is amplified and detected via real time PCR.

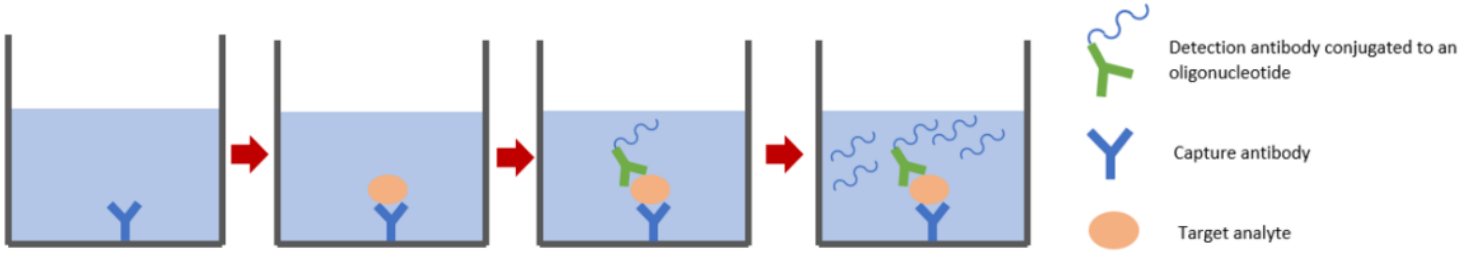


Figure 3. Schematic representation of the steps of an immuno-PCR assay.

Advantages of immuno-PCR for immunodetection:

- Can detect any protein.
- Provides exponential signal amplification.
- Highly sensitive – can detect extremely low amounts of analyte (pg – fg).
- Fewer incubation steps so improved assay reproducibility.
- Provides the option of multiplexing.
- Needs only small sample volumes.
- Compatible with complex samples.

Despite the advantages of immuno-PCR, an ELISA continues to be the primary method of immunodetection. This is, in part, because the conjugation of antibodies to oligonucleotides has traditionally been associated with requiring special expertise and being an expensive and time-consuming process.

Proximity Ligation Assay

Promixity Ligation Assay (PLA) was developed by Fredriksson and colleagues in 2002 and has since become commercialized by Olink Bioscience.[2] It is a immunohistochemical tool used for highly sensitive and specific protein analysis. Similar to immuno-PCR, Proximity Ligation Assay combines the specificity of an ELISA with the sensitivity of PCR.

How PLA works

A typical Proximity Ligation Assay uses two primary antibodies, each raised in different species for the detection of two unique targets, and two oligo-conjugated secondary antibodies (PLA probes) which bind to the primaries. An additional oligonucleotide is introduced, and if the two PLA probes are in close proximity, which occurs if the target proteins are adjacently present, then the free ends of the PLA probes become ligated by DNA ligase. This leads to rolling circle amplification (RCA), resulting in a 1000-fold amplification which can be quantified by real-time PCR.

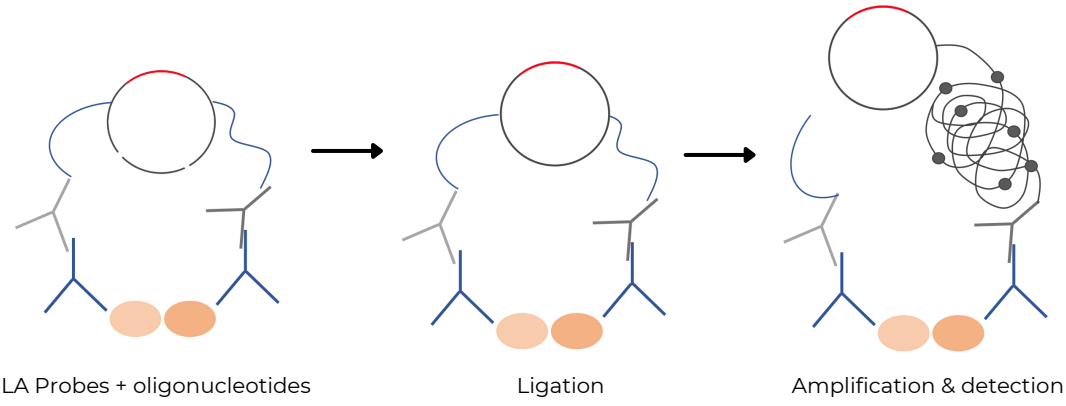


Figure 4. Indirect Proximity Ligation Assay. Directly conjugated primary antibodies can also be used for PLA.

Proximity Ligation Assay provides several advantages including being quick and easy to perform, being highly sensitive and specific, producing reliable results, and having the potential for multiplexing. However there are some limitations to the application, in particular high background from non-specific ligation, and the time, cost and expertise required to conjugate the antibody to the oligonucleotide to form the PLA probes.

2 The challenges and limitations of oligo conjugation

As previously discussed, antibody-oligonucleotide conjugation is notoriously a complex and time-consuming process; however there are now several technologies available to scientists that claim to make the process of oligo conjugation quick and easy. Despite these advantages, many of these conjugation methods are based in chemical approaches that have numerous shortcomings concerning conjugation efficiency. These include:

- **The requirement for excess oligonucleotide reactants**

To achieve a high percentage of conjugated antibody with oligos, chemical-based kits often require a high concentration and large excess of the oligonucleotide reactant. With the production of custom oligonucleotides being an expensive and time-consuming process, the waste of precious oligo material is a common source of frustration for scientists working with oligo conjugates as part of their research.

> **Key Takeaway:** Researchers should not have to waste their money or time by mixing a large excess of oligo to achieve successful conjugations.

- **The requirement for high antibody concentrations**

The competing chemical-based kits often require high concentrations of antibodies for successful conjugation. This often requires concentrating the antibody prior to conjugation, which can result in antibody loss and wasted time. Using high amounts of antibody for a single conjugation reaction is also wasteful since such large amounts of antibody are often not required for sensitive antibody-oligo applications.

> **Key Takeaway:** Researchers should not have to worry about a minimum amount of antibody needed for a reaction when conjugating to their oligos.

- **Protocols are complex and require troubleshooting**

Many oligonucleotide conjugation kits claim to solve the issues of needing an in-house conjugation expert. However the protocols can often be long (5-6 hours hands on time) and require ongoing troubleshooting, taking up precious time and resources with the potential for an unsuccessful outcome. It can also lead to batch to batch variants of conjugates.

> **Key Takeaway:** Researchers should be able to achieve reliable conjugation every time. Troubleshooting should be a biology question, not a reagent question!

- **Uncontrolled conjugation can lead to variable binding**

Many commercial oligonucleotide labeling technologies utilize lysine (amine) or cysteine directed conjugation which can result in variable binding of oligo to the antibody, as well as the potential for binding in the antibody binding site.

> **Key Takeaway:** Site-directed and controlled conjugation is necessary to avoid variability.

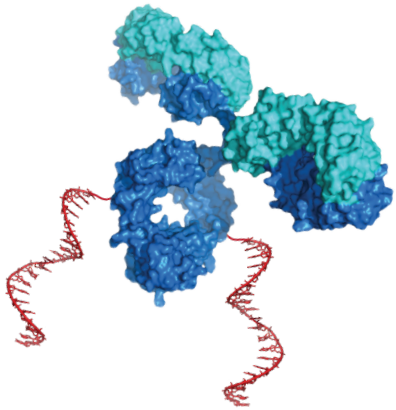
- **Buffer exchange can lead to antibody loss**

Chemical-based conjugation kits often cannot be performed in the presence of antibody storage buffers that contain Tris or storage proteins such as bovine serum albumin. Therefore, these antibodies must be buffer exchanged into a compatible buffer before being conjugated with oligonucleotides, which can result in antibody loss and time wasted.

> **Key Takeaway:** Researchers should not have exchange the antibody buffer prior to a conjugation reaction.

2 The technology helping researchers overcome conjugation challenges

Imagine if you could site-specifically label your antibody with custom oligos, using only the amount of antibody you need for your experiment and without a large excess of oligo.



With oYo-Link® this is possible!

oYo-Link® Oligo enables rapid, site-specific labeling of compatible antibodies with both ssDNA & dsDNA oligonucleotides, up to 80bp or longer. The technology enables labeling with only 30 seconds hands-on time, and the whole conjugation process is complete within 2 hours.

- Label as little as 1ug of antibody at a time – reduce overall antibody required = reduce overall cost and minimize wasted materials!
- Custom oligos supported, up to 80bp or longer, for ssDNA or dsDNA.
- Easy and rapid oligo-antibody labeling with less than 30 seconds hands-on time.
- Site specific labeling in the Fc region, so no loss in antibody functionality or batch-to-batch variability.
- Oligos are all HPLC or PAGE grade ensuring confidence in downstream assays.
- Label diluted antibody – as low as 50 µg/mL – saving time as no need to concentrate your antibody prior to labeling.
- No need for purification prior to conjugation - compatible with nearly all buffers, even those containing Tris and albumin.

Unlike other oligonucleotide conjugation products available, oYo-Link allows site specific, covalent labeling of antibodies in the Fc region, assuring there are 1 to 2 oligonucleotides attached per antibody and yielding a homogenous mixture of antibody-oligo conjugates.

Therefore, oYo-Link Oligo provides scientists with more control and precision over their experiments, removing any impact of decreased assay performance associated with traditional heterogeneous labeling products.

How does oYo-Link® technology work?

oYo-Link® reagents consist of low molecular weight (~8 kDa), high-affinity antibody-binding domains that possess a photo-crosslinker within their Fc-binding site. Upon illumination with non-damaging Black-light, oYo-Link forms a covalent bond with the antibody. This procedure is referred to as Light-Activated Site-Specific Conjugation (LASIC). Any label that is attached to oYo-Link will be covalently attached to the desired antibody.

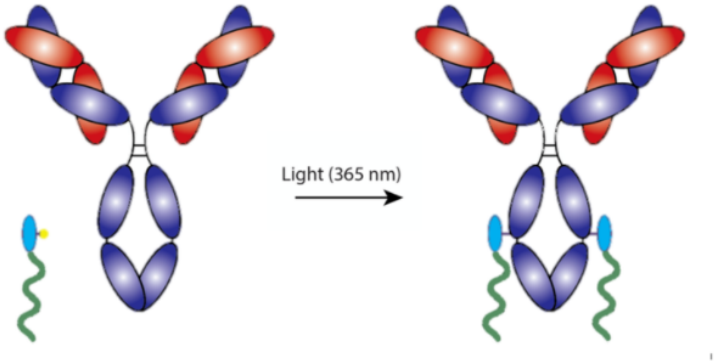


Figure 5. The oYo-Link site-specific labeling process attaches 1-2 oligos to the Fc region of the antibody using non-damaging UV light.

Labeling is extremely quick with only 30 seconds hands-on time required and 2 hours total conjugation time. Simply add oYo-Link to your antibody, illuminate for 2 hours and your antibody will be covalently labeled with your oligonucleotide and ready to use! There are no purification steps required post labeling, meaning 100% sample recovery.
You just provide us with your oligo sequence and we send you the custom oYo-Link Oligo reagent ready to use with compatible antibodies.

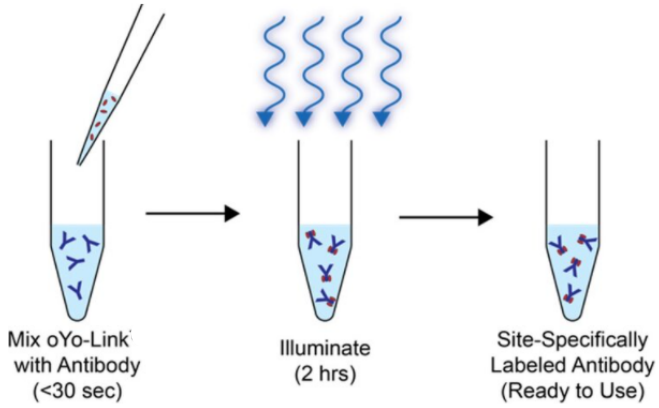


Figure 6. The oYo-Link Oligo conjugation process requires only 2 steps and 30 seconds hands-on time. Conjugates are ready to use after 2 hours.

All Oligonucleotides are of the highest quality, manufactured by Integrated DNA Technologies, Inc.

oYo-Link® Oligo Crosslinking data

The superior conjugation efficiency is demonstrated below for oligos of varying lengths and sequence. The shift in the bands indicates high conjugation efficiency with mixing 1uL of the oYo-Link Oligo reagents with only 1ug of antibody.

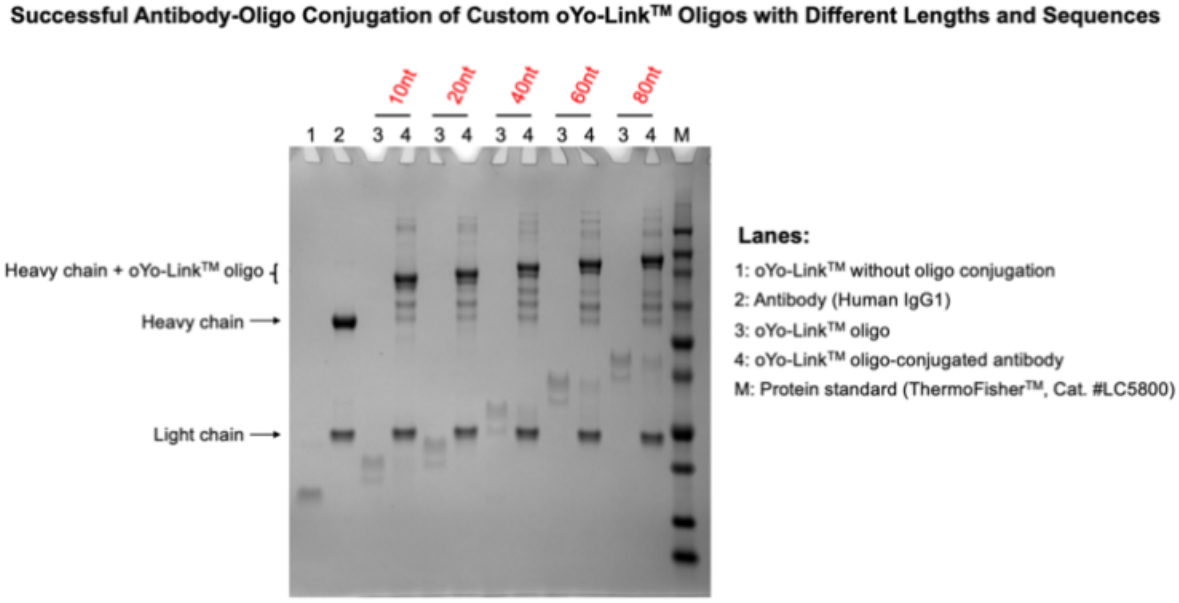


Figure 7. The successful antibody-oligo conjugation of oYo-Link Oligo Custom for Oligos of Varying Length & Sequence: oYo-Link allows efficient oligo-antibody conjugation using custom oligos with different sequences and length: 1µL each of the oYo-Link Oligo reagents was mixed with 1µg of antibody (human IgG1) respectively and photo-crosslinked under 365nm UV for 2hrs. Each of the five oligos has a different sequence and a different length, varying from 10nt to 80nt. Samples were reduced and run on a 4-12% Bis-Tris denaturing gel. Following site-specific photo-crosslinking of the antibody's heavy chain with oYo-Link Oligo, an upward shift of the antibody heavy chain was observed. The figure above demonstrates over 90% conjugation efficiency of oYo-Link Oligo to the antibody. There was no significant conjugation efficiency difference observed among oligos with different sequences and lengths.

Want to know more?

Download our brochure to find out more about oYo-Link Oligo Custom, how it can benefit you and how to order. Plus receive 20% discount on your first order with us!

Or if you have any questions about the technology get in touch with our technical support team.

References

[1] Chung et al. (2021). Joint single-cell measurements of nuclear proteins and RNA in vivo. *Nat Methods*, 18(10):1204-1212. doi: 10.1038/s41592-021-01278-1.

[2] Fredriksson et al. (2002). Protein detection using proximity-dependent DNA ligation assays. *Nat Biotechnol*, 20(5):473-7. doi: 10.1038/nbt0502-473

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