Service Sheet

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Generation of sdAb-oligonucleotide conjugates

Oligonucleotides

Single-domain antibodies (sdAbs) are valuable tools for detecting proteins protein-protein interactions. Because of their small size, labeled sdAbs cause a small distance between target protein and label, which is referred to as small linkage error.[1]

Besides fluorophores, biotin, or enzymes, oligonucleotides have proven to be valuable labels in biophysical assays. In the form of single- or double-stranded pieces of DNA or RNA, oligonucleotides can be conjugated to various targeting probes.[2,3,4] In such biophysical assays, targeted oligonucleotides can undergo hybridization and ligation (in e.g. PLA) or extension (in e.g. PEA) and often amplification, making the assay highly sensitive. Subsequently, the probes can be detected using qPCR or labeled complementary oligonucleotides, either followed by direct quantification or (high-resolution) microscopy imaging.[3,4,5] In particular, the very small sdAb-oligo conjugates (Figure 1) allow for the sensitive detection of proteins and protein-protein interactions with a low linkage error.

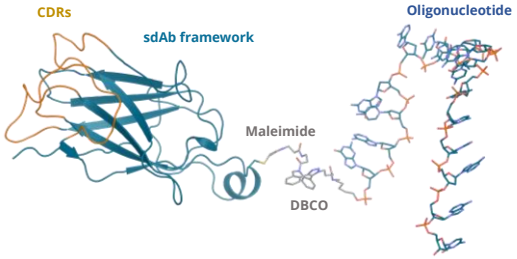


Figure 1. Structure model of sdAb-oligo. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine and DBCO to single-stranded oligonucleotide.

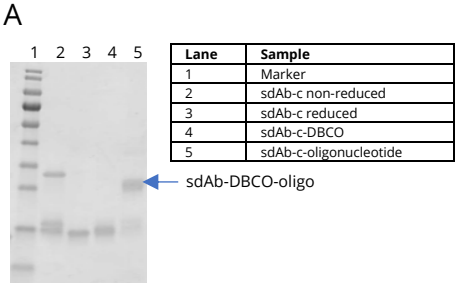
sdAb-oligonucleotide conjugation

SdAb-oligonucleotide conjugates are generated by using maleimide-DBCO as bifunctional linker between an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and an azide-modified oligonucleotide. The resulting conjugate is checked for protein integrity, degree of labeling, and target binding (Figure 2). QVQ conjugates off-the-shelf sdAb products, as well as your sdAb of choice, to custom-ordered oligonucleotides.

Deliverables

- Oligonucleotide-labeled sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration, degree of labeling
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity of the conjugate (ELISA detected with antisense-oligo)

Examples:



Lane	Sample
1	Marker
2	sdAb-c non-reduced
3	sdAb-c reduced
4	sdAb-c-DBCO
5	sdAb-c-oligonucleotide

Figure 2. Example of quality control of generated sdAb-oligonucleotide conjugate. A) SDS PAGE after conjugation of azide-modified oligonucleotide to sdAb-DBCO. B) ELISA measuring binding of sdAb-oligonucleotides targeting CXCR4 to CXCR4-expressing HeLa cells. Binding of conjugate is detected by complementary DNA strand conjugated to biotin and streptavidin-HRP.

References:

1 Ries et al (2012) Nat. Methods 9, 582-584.
2 Gong et al. (2015) Bioconjugate Chem. 27(1), 217-225.
3 Al-Amim et al (2022) Anal. Chem. 94(28), 10054-10061.
4 Söderberg et al (2008) Methods 45(3), 227-232.
5 Dovgan et al (2019) Bioconjugate Chem 30(10), 2483-2501.