Service Sheet

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Generation of sdAb-DBCO conjugates for click chemistry

sdAb conjugation

sdAbs can be easily functionalized by attaching labels, typically through NHS labeling of amines of lysines or site-directed thiol-maleimide coupling of cysteines. As lysines in sdAbs often contribute to their target binding, site-directed conjugation is the preferred approach.[1] For many labels, thiol-reactive maleimide-modified variants are commercially available. However, if these are not available, azide-modified labels can be a viable alternative as they react readily with dibenzocyclooctyne (DBCO).[2] In that case, sdAb-DBCO conjugates (Figure 1) are used as intermediate reagents.

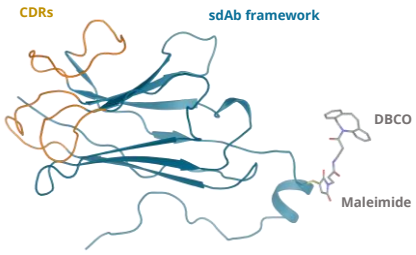


Figure 1. Structure model of sdAb-DBCO. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine to maleimide-DBCO.

DBCO

DBCO, also known as Dibenzoazacyclooctyne (DIBAC), is a molecule comprising two benzene rings and a cyclooctyne ring.[3] This 8-membered ring contains a C-C triple bond, which introduces significant strain and makes the molecule highly reactive.[3,4] In strain-promoted azide-alkyne cycloaddition (SPAAC) reactions, DBCO reacts readily with azides (Figure 2).[2,4] This reaction is a classic example of copper-free click chemistry and is widely used in bioconjugations covalently linking two molecules.[5,6]

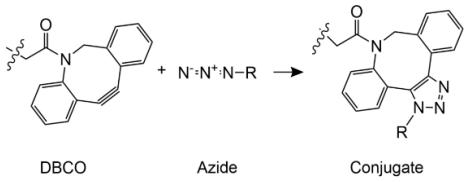


Figure 2. Reaction of DBCO and azide. DBCO (conjugated to sdAb) and azide (attached to label) undergo strain-promoted alkyne-azide cycloaddition (SPAAC) forming sdAb-label conjugate.

sdAb-DBCO conjugates allow the incorporation of azide-modified molecules, such as fluorophores, peptides, oligonucleotides, and more (Figure 3).

Deliverables

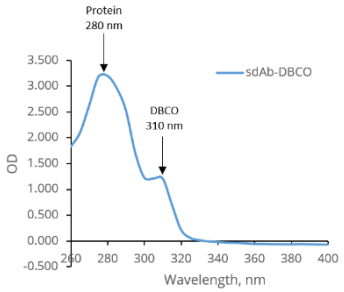
- DBCO-labeled sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration, degree of labeling
  - UV-Vis spectrum
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity (ELISA)

sdAb-DBCO conjugation

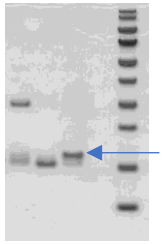
QVQ generates sdAb-DBCO conjugates via a copper-free click reaction of an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and maleimide-modified DBCO. The resulting conjugate is checked for protein integrity, degree of labeling, and target binding (Figure 3).

Examples:

A

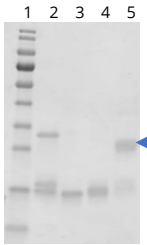


B



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

C



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

D

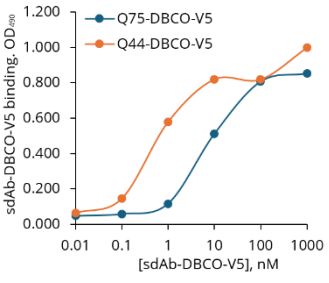


Figure 3. Example of quality control of generated sdAb-DBCO conjugates. A) UV-Vis spectrum for calculation of degree of labeling (DOL). B-C) SDS PAGE after conjugation of azide-modified peptide (B) or oligonucleotide (C) to sdAb-DBCO. D) sdAbc-DBCO-V5 tag conjugates binding to recombinant protein targets in ELISA. Bound V5-tagged sdAbs are detected using anti-V5-tag antibody.

References:

1 Massa et al (2014) Bioconjugate Chem. 25(5), 979-988.
2 Shi et al (2020) Angew. Chem. Int. 59(45), 19940-19944.
3 He and Cheng (2023) Molecules 28(9), 3715.
4 Albada et al (2021) Chem Rev 121, 7032-7058.
5 Wiener et al. (2020) Sci Rep 10, 1457.
6 Gong et al (2016) Bioconjugate Chem. 27(1), 217-225.