

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.

DBCO

DBCO, also known as Dibenzozacyclooctyne (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}

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

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:

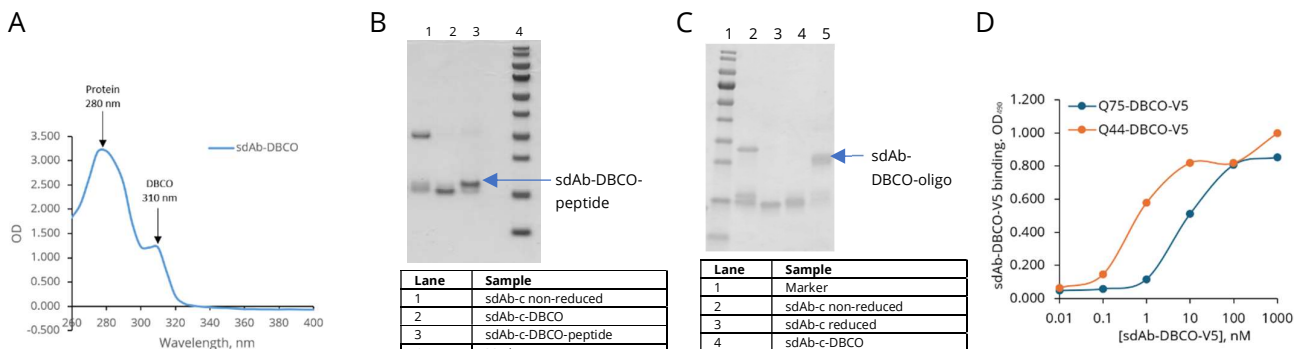


Figure 3. Example of quality control of generated sdAb-DBCO conjugates. A) UV-Vis spectrum for calculation of degree of labeling (DOL). B-C) SDS PAGE after conjugation of azide-modified peptide (B) or oligonucleotide (C) to sdAb-DBCO. D) sdAb-DBCO-V5 tag conjugates binding to recombinant protein targets 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.