DBCO Azide Ligation

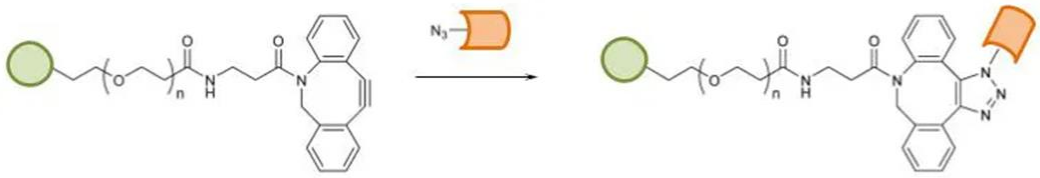

Fig 1. Schematic representation of a SPAAC ligation reaction

Condition1: EtOH/ H_2O (3:2), room temperature

Condition2: DMSO/H_2O, room temperature

Dibenzocyclooctyne(DBCO) reagent is a class of click chemistry labeling reagents. DBCO group can exclusively react with azide-tagged molecules or biomolecules to form a stable triazole. The click chemistry is also known as strain promoted alkyne-azide cycloaddition (SPAAC), DBCO reagent has become widely used in bioconjugation, labeling and chemical biology.

DBCO click chemistry can be run in aqueous buffer or in organic solvents depending on the property of the substrate molecules. Reagents with PEG arm will increases the compound's hydrophilicity.

This DBCO-Azide method requires to activate the biomolecule #1 with DBCO reagent, and the biomolecule #2 with azide, then to mixing the two activated biomolecules to form a conjugate.

Features

- Biocompatibility – no cytotoxic Copper catalyst required – Nice of in-vivo applications.
- Mild conditions – conjugation in aqueous buffered media and at low temperature
- Stability – DBCO and azide moieties are long term stable
- Efficiency – formation of a stable triazole in quantitative yield
- Specificity and Bioorthogonality azide react only with DBCO in the presence of -NH2, -SH, -COOH and other protein functional group
  Note: NaN3 will react with DBCO.
- Reaction traceable by UV – Vis spectroscopy, DBCO absorption at 310nm

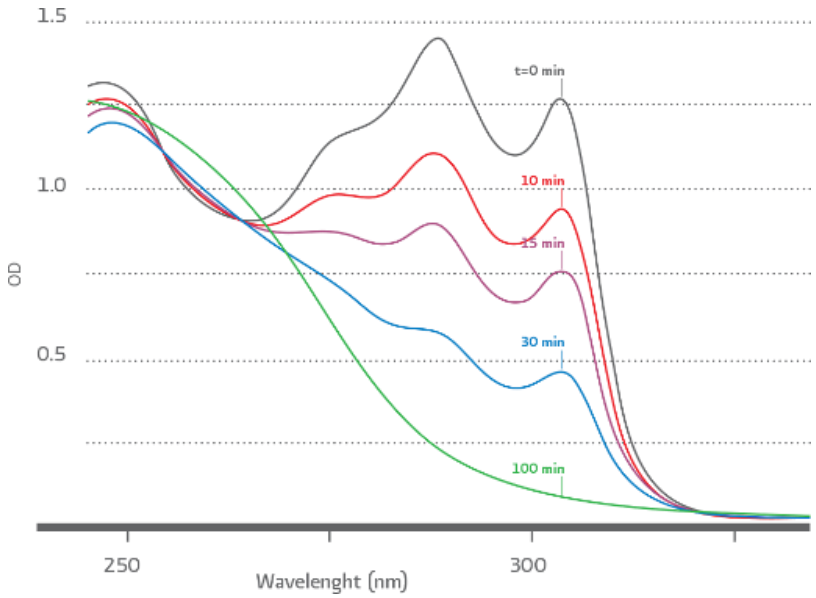

Fig 2 Progress of SPAAC ligation reaction followed by UV–Vis spectroscopy

Example protocol: (Antibody-oligo conjugation)

Antibody activation

- Mix antibody with 20-30 fold molar excess of DBCO NHS ester dissolved in DMSO (10 mM solution). DMSO content in the final mixture should be around 20%, antibody concentration in the reaction mixture around 1 mg/mL.
- Incubate at room temperature for 60 minutes.
- Add Tris (10 uL, 100mM in water) to the reaction to quench the unreacted DBCO-NHS ester.
- Incubate for 15 minutes.
- Remove the unreacted DBCO-NHS ester using spin desalting column.

Note: DBCO-functionalized antibody can be stored at -20°C for up to month, but DBCO functional group loses its reactivity over the time due to its oxidation and addition of water to the triple bond.

Characterization of DBCO-Antibody Conjugates

The average number of conjugated DBCO molecules per antibody (DBCO/Ab) can be determined by the equation below.

$$\frac{\text{DBCO}}{\text{Ab}} = \frac{\text{A309} \times \varepsilon_{\text{Ab}}}{(\text{A280} - \text{CF} \times \text{A309}) \times \varepsilon_{\text{DBCO}}}$$

Where

ε_{DBCO}: the molar extinction coefficients of the DBCO at 309nm; 12,000 M^{-1} cm^{-1},
ε_{Ab}: the molar extinction coefficients of IgG antibody at 280 nm: 204,000 M^{-1} cm^{-1},
A309: is the absorption value of the sample at 309 nm.

A280: the absorption value of the sample at 280nm
CF: the correction factor of DBCO at 280 nm; 1.089

Click Chemistry

- Mix DBCO-functionalized antibody or another biomolecule with 2-4x molar excess of azide modified oligonucleotide or azide functionalized dye.
- Incubated overnight at 4°C.
- Validate your final conjugate using SDS gel electrophoresis
- Remove the unreacted oligonucleotide or dye using liquid chromatography (reverse phase HPLC, ion exchange HPLC, or both).

Reference

- Sachin, Kalme et al., Bioconjugate Chemistry, 23(8), 1680-1686; 2012.