

# DBCO–Azide Conjugation

## OVERVIEW

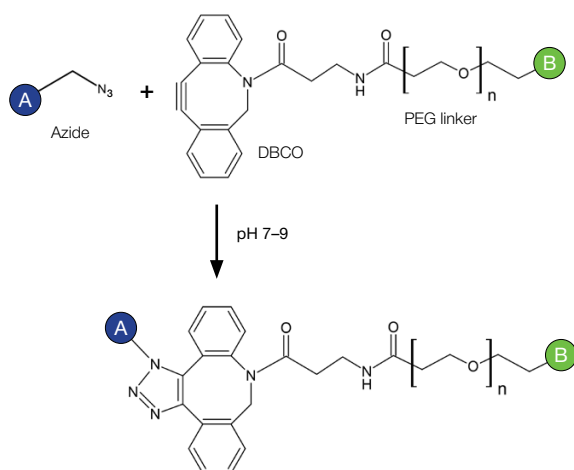
**Dibenzocyclooctyne (DBCO)** reagents enable bioorthogonal “click” reactions with azide-functionalized molecules through Strain-Promoted Alkyne–Azide Cycloaddition (SPAAC). This copper-free reaction forms a stable triazole product under mild conditions, making it ideal for bioconjugation, labeling, and chemical biology applications.

### Key Features

- **Biocompatible:** No cytotoxic copper catalyst required (suitable for in vivo applications).
- **Mild Conditions:** Efficient conjugation in aqueous buffer at low or room temperature.
- **Long-Term Stability:** Both DBCO and azide moieties remain stable for extended periods.
- **High Efficiency:** Triazole formation typically proceeds in quantitative yield.
- **Specific & Bioorthogonal:** Azide reacts only with DBCO in the presence of other functional groups (e.g.,  $-\text{NH}_2$ ,  $-\text{SH}$ ,  $-\text{COOH}$ ).
- **UV Traceability:** DBCO absorbs strongly at  $\sim 310$  nm, allowing reaction monitoring by UV–Vis.

### Notes

- **Solvent Conditions:** Although DBCO–Azide reactions can proceed in partially organic media (e.g., up to 20% DMSO in aqueous buffer), for antibody conjugations we typically recommend standard aqueous buffers (e.g., PBS) without sodium azide, since azide will deplete DBCO.
- **PEG Linkers:** DBCO or azide reagents containing PEG arms offer enhanced hydrophilicity.



**Figure 1.** Schematic of SPAAC (Strain-Promoted Azide-Alkyne Cycloaddition) ligation reaction, in which a triazole bond is formed between an azide molecule and DBCO with high efficiency and specificity under mild conditions.

## GENERAL LIGATION WORKFLOW

1. **Azide Activation of Biomolecule A**
  - Attach an azide moiety to Biomolecule A.
2. **DBCO Activation of Biomolecule B**
  - Separately, conjugate a DBCO reagent (e.g., DBCO–NHS ester) to Biomolecule B.
3. **Click Reaction**
  - Mix the DBCO-functionalized Biomolecule #1 with the azide-functionalized Biomolecule #2 to form the stable triazole conjugate.

## EXAMPLE PROTOCOL: ANTIBODY–OLIGO CONJUGATION

### Antibody Activation with DBCO

1. **Reaction Setup**
  - Dissolve DBCO–NHS ester in DMSO at 10 mM.
  - Use antibody at 1–10 mg/mL in a suitable buffer (e.g., PBS, pH  $\sim 7.4$ ). (In many SE-labeling protocols, 2 mg/mL or higher is standard; a range of 1–10 mg/mL is acceptable.)
  - Mix the antibody with a 20–30-fold molar excess of DBCO–NHS.
  - Keep the final DMSO content at less than 20%.
2. **Incubate**
  - React at room temperature for 60 minutes.
3. **(Optional) Quench**
  - Typically, SE conjugations do not require a specific quench step and proceed directly to purification. However, if desired, add  $\sim 10$   $\mu\text{L}$  of 100 mM glycine (in water) to neutralize unreacted DBCO–NHS.
  - Incubate for 15 minutes.

### Characterize the DBCO–Antibody (Degree of Substitution)

To calculate the average number of DBCO molecules per antibody (sometimes referred to as DOS or DBCO/Ab), measure the absorbance of the purified conjugate at 280 nm and 309 nm and use the following formula:

$$\text{DBCO} / \text{Ab} = \frac{A_{309} \times \epsilon_{\text{Ab}}}{A_{280} - (\text{CF} \times A_{309}) \times \epsilon_{\text{DBCO}}}$$

Where:

- $\epsilon_{\text{DBCO}} = 12,000 \text{ M}^{-1} \text{ cm}^{-1}$  at 309 nm
- $\epsilon_{\text{Ab}} = 210,000 \text{ M}^{-1} \text{ cm}^{-1}$  at 280 nm (for IgG)
- $A_{309}$  = absorbance at 309 nm
- $A_{280}$  = absorbance at 280 nm
- CF = 0.955 (correction factor for DBCO absorbance bleed at 280 nm)

**EXAMPLE PROTOCOL: ANTIBODY-OLIGO CONJUGATION CONT.**

Click Chemistry with Azide-Oligonucleotide

1. Set Up Reaction

- Mix the DBCO-antibody with a 2-4-fold molar excess of azide-modified oligonucleotide (or azide-labeled dye).
- Recommended solvent: PBS or similar buffer with up to 20% DMSO. Avoid sodium azide.

2. Incubate

- Incubate 2-4 hours at room temperature (or overnight at 4 °C) to ensure complete reaction.

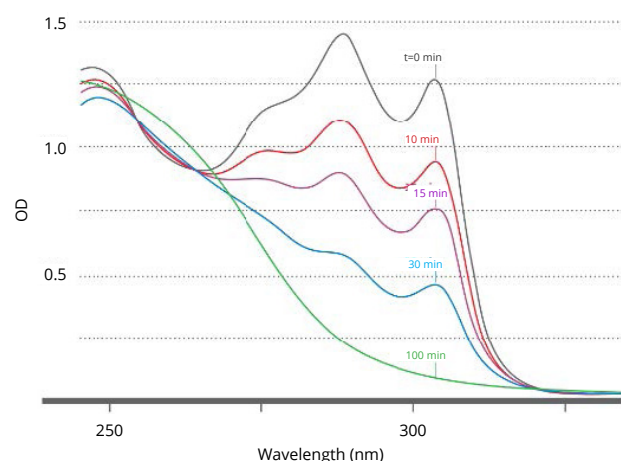
3. Validation

- Check conjugate formation via SDS-PAGE. The antibody-oligo conjugate should show a higher molecular weight band than the unmodified antibody.

4. Purification

- Remove excess oligonucleotide or dye by liquid chromatography (e.g., reverse-phase or ion-exchange HPLC) or another appropriate method.

**EXAMPLE DATA ANALYSIS AND FIGURES**



**Figure 2.** Real-time UV-Vis monitoring of the SPAAC (DBCO-Azide) reaction at various time points (0-100 min). The initial black spectrum (t=0 min) shows a pronounced DBCO peak near 310 nm, which progressively diminishes as the reaction proceeds. By 100 min (green line), the 310 nm peak is significantly reduced, indicating extensive consumption of DBCO.

**APPENDIX**

**Table 1.** Ordering information for AAT Bioquest azide and DBCO products

| Cat. No. | Product                         | Quantity |
|----------|---------------------------------|----------|
| 12642    | 10XHis azide                    | 1 mg     |
| 20332    | 2',3'-cGAMP azide               | 100 µg   |
| 20330    | 2',3'-cGAMP-DBCO conjugate      | 100 µg   |
| 21243    | 5-(and-6)-Carboxy SNARF-1 Azide | 1 mg     |
| 131      | 5-FAM Azide                     | 10 mg    |
| 950      | 5-FAM Azide                     | 100 mg   |
| 486      | 5-TAMRA azide                   | 5 mg     |
| 960      | 5-TAMRA azide                   | 50 mg    |
| 133      | 6-FAM Azide                     | 10 mg    |
| 955      | 6-FAM Azide                     | 100 mg   |
| 240      | 6-HEX azide                     | 5 mg     |
| 248      | 6-JOE azide                     | 5 mg     |
| 217      | 6-NED azide                     | 1 mg     |
| 236      | 6-OG488 Azide                   | 1 mg     |
| 494      | 6-ROX azide                     | 5 mg     |
| 490      | 6-TAMRA azide                   | 5 mg     |
| 965      | 6-TAMRA azide                   | 50 mg    |
| 244      | 6-TET azide                     | 5 mg     |
| 219      | 6-VIC Azide                     | 1 mg     |
| 12628    | 6XHis azide                     | 1 mg     |
| 12623    | 6XHis-DBCO Conjugate            | 1 mg     |
| 26019    | Acridinium Azide                | 1 mg     |
| 508      | AMCA Azide                      | 1 mg     |
| 70203    | AATOM™ 390 azide                | 1 mg     |
| 70205    | AATOM™ 390 DBCO                 | 1 mg     |
| 70213    | AATOM™ 425 azide                | 1 mg     |
| 70215    | AATOM™ 425 DBCO                 | 1 mg     |
| 2817     | AATOM™ 488 azide                | 1 mg     |
| 2819     | AATOM™ 488 DBCO                 | 1 mg     |
| 2802     | AATOM™ 488 PEG3 azide           | 1 mg     |
| 70223    | AATOM™ 495 azide                | 1 mg     |
| 70225    | AATOM™ 495 DBCO                 | 1 mg     |
| 2845     | AATOM™ 514 azide                | 1 mg     |
| 2847     | AATOM™ 514 PEG4 DBCO            | 1 mg     |
| 2824     | AATOM™ 532 PEG3 azide           | 1 mg     |
| 2821     | AATOM™ 532 PEG4 DBCO            | 1 mg     |
| 70233    | AATOM™ 550 azide                | 1 mg     |
| 70235    | AATOM™ 550 DBCO                 | 1 mg     |
| 70243    | AATOM™ 590 azide                | 1 mg     |
| 70245    | AATOM™ 590 DBCO                 | 1 mg     |
| 2840     | AATOM™ 594 PEG3 azide           | 1 mg     |
| 2842     | AATOM™ 594 PEG4 DBCO            | 1 mg     |
| 70253    | AATOM™ 610 azide                | 1 mg     |

APPENDIX CONT.

| Cat. No. | Product  | Quantity |
|----------|--|----------|
| 70255    | AATOM™ 610 DBCO                                | 1 mg     |
| 70263    | AATOM™ 620 azide                               | 1 mg     |
| 70265    | AATOM™ 620 DBCO                                | 1 mg     |
| 70273    | AATOM™ 633 azide                               | 1 mg     |
| 70275    | AATOM™ 633 DBCO                                | 1 mg     |
| 2832     | AATOM™ 647 PEG4 DBCO                           | 1 mg     |
| 2835     | AATOM™ 647N azide                              | 1 mg     |
| 2837     | AATOM™ 647N DBCO                               | 1 mg     |
| 2806     | AATOM™ 647N PEG3 azide                         | 1 mg     |
| 2807     | AATOM™ 647N PEG4 DBCO                          | 1 mg     |
| 70283    | AATOM™ 655 PEG3 azide                          | 1 mg     |
| 70285    | AATOM™ 655 PEG4 DBCO                           | 1 mg     |
| 70293    | AATOM™ 680 PEG3 azide                          | 1 mg     |
| 70295    | AATOM™ 680 PEG4 DBCO                           | 1 mg     |
| 70303    | AATOM™ 700 PEG3 azide                          | 1 mg     |
| 70305    | AATOM™ 700 PEG4 DBCO                           | 1 mg     |
| 2412     | AzoDye-1 Azide                                 | 1 mg     |
| 2413     | AzoDye-1-PEG10-Azide                           | 1 mg     |
| 2432     | AzoDye-2 Azide                                 | 1 mg     |
| 2431     | AzoDye-2 C2 Azide                              | 1 mg     |
| 2433     | AzoDye-2-PEG10 Azide                           | 1 mg     |
| 2482     | AzoDye-3 Azide                                 | 1 mg     |
| 2483     | AzoDye-3 PEG10 Azide                           | 1 mg     |
| 20423    | BAPTA Azide                                    | 1 mg     |
| 20425    | BAPTA DBCO                                     | 1 mg     |
| 12568    | BG Azide                                       | 1 mg     |
| 3023     | Biotin Azide                                   | 5 mg     |
| 3020     | Biotin C2 Azide                                | 5 mg     |
| 3025     | Biotin-PEG2-azide *CAS 945633-30-7*            | 5 mg     |
| 3019     | Biotin-PEG3-azide *CAS 875770-34-6*            | 5 mg     |
| 20613    | Cal-520® azide                                 | 100 µg   |
| 6909     | CDPI3 Azide [Minor Groove Binder Azide]        | 1 mg     |
| 943      | Cy3B azide                                     | 1 mg     |
| 945      | Cy3B DBCO                                      | 1 mg     |
| 143      | Cyanine 3 azide [equivalent to Cy3® azide]     | 1 mg     |
| 970      | Cyanine 3 azide [equivalent to Cy3® azide]     | 25 mg    |
| 980      | Cyanine 3.5 azide [equivalent to Cy3.5® azide] | 1 mg     |
| 981      | Cyanine 3.5 azide [equivalent to Cy3.5® azide] | 25 mg    |
| 153      | Cyanine 5 azide [equivalent to Cy5® azide]     | 1 mg     |
| 975      | Cyanine 5 azide [equivalent to Cy5® azide]     | 25 mg    |

| Cat. No. | Product  | Quantity |
|----------|--|----------|
| 178      | Cyanine 5.5 azide [equivalent to Cy5.5® azide] | 1 mg     |
| 163      | Cyanine 7 azide [equivalent to Cy7® azide]     | 1 mg     |
| 2010     | DABCYL-DBCO                                    | 5 mg     |
| 4529     | DBCO NHS Ester                                 | 10 mg    |
| 4534     | DBCO PEG4 Amine                                | 25 mg    |
| 4527     | DBCO Sulfo-NHS Ester                           | 5 mg     |
| 4528     | DBCO Sulfo-NHS Ester                           | 25 mg    |
| 920      | DBCO-Cy3                                       | 1 mg     |
| 923      | DBCO-Cy5                                       | 1 mg     |
| 4530     | DBCO-PEG4-NHS Ester                            | 1 mg     |
| 4526     | DBCO-PEG5-NHS Ester                            | 1 mg     |
| 3504     | Digoxigenin azide                              | 1 mg     |
| 72710    | FastClick™ 5-FAM Azide                         | 1 mg     |
| 72712    | FastClick™ 5-TAMRA Azide                       | 1 mg     |
| 72711    | FastClick™ 6-FAM Azide                         | 1 mg     |
| 72714    | FastClick™ 6-ROX Azide                         | 1 mg     |
| 72713    | FastClick™ 6-TAMRA Azide                       | 1 mg     |
| 72801    | FastClick™ Biotin Azide                        | 1 mg     |
| 72700    | FastClick™ Cy3 Azide                           | 1 mg     |
| 72702    | FastClick™ Cy5 Azide                           | 1 mg     |
| 72704    | FastClick™ Cy7 Azide                           | 1 mg     |
| 72800    | FastClick™ Digoxigenin (DIG) Azide             | 1 mg     |
| 72730    | FastClick™ XFD350 Azide                        | 1 mg     |
| 72733    | FastClick™ XFD405 Azide                        | 1 mg     |
| 72735    | FastClick™ XFD488 Azide                        | 1 mg     |
| 72737    | FastClick™ XFD555 Azide                        | 1 mg     |
| 72740    | FastClick™ XFD647 Azide                        | 1 mg     |
| 72745    | FastClick™ XFD750 Azide                        | 1 mg     |
| 17616    | Helixyte™ Green Azide                          | 1 mg     |
| 17674    | Hoechst 33342 azide                            | 1 mg     |
| 985      | ICG azide                                      | 1 mg     |
| 1094     | iFluor® 405 azide                              | 1 mg     |
| 1000     | iFluor® 488 azide                              | 1 mg     |
| 1093     | iFluor® 555 azide                              | 1 mg     |
| 1095     | iFluor® 594 azide                              | 1 mg     |
| 71800    | iFluor® 625 azide                              | 1 mg     |
| 1091     | iFluor® 647 azide                              | 1 mg     |
| 71801    | iFluor® 720 Azide                              | 1 mg     |
| 1363     | iFluor® 790 Azide                              | 1 mg     |
| 20342    | IP1 Azide                                      | 100 µg   |
| 50637    | Isotonitazene Azide                            | 1 mg     |
| 832      | Methylene Blue Azide                           | 1 mg     |
| 1690     | mFluor™ Violet 450 Azide                       | 1 mg     |
| 12605    | NTA Azide                                      | 1 mg     |
| 5303     | Phalloidin-PEG4-DBCO                           | 100 µg   |

APPENDIX CONT.

| Cat. No. | Product   | Quantity |
|----------|---|----------|
| 39057    | Psoralen MOP Azide  | 1 mg     |
| 39062    | Psoralen TMP Azide  | 1 mg     |
| 3403     | ReadiLeave™ Reversible Biotin Azide                             | 1 mg     |
| 16883    | ReadiUse™ Preactivated Streptavidin Azide                       | 1 mg     |
| 21125    | Rhod-4™ azide   | 100 µg   |
| 39004    | SDA azide   | 1 mg     |
| 484      | Texas Red® azide *Single Isomer*                                | 5 mg     |
| 2236     | Tide Fluor™ 1 azide [TF1 azide]                                 | 5 mg     |
| 2252     | Tide Fluor™ 2 azide [TF2 azide]                                 | 1 mg     |
| 2254     | Tide Fluor™ 3 azide [TF3 azide]                                 | 1 mg     |
| 2300     | Tide Fluor™ 4 azide [TF4 azide]                                 | 1 mg     |
| 2275     | Tide Fluor™ 5WS azide [TF5WS azide]                             | 1 mg     |
| 2302     | Tide Fluor™ 6WS azide [TF6WS azide]                             | 1 mg     |
| 2304     | Tide Fluor™ 7WS azide [TF7WS azide]                             | 1 mg     |
| 2306     | Tide Fluor™ 8WS azide [TF8WS azide]<br>*Near Infrared Emission* | 1 mg     |
| 2188     | Tide Quencher™ 1 azide [TQ1 azide]                              | 5 mg     |
| 2211     | Tide Quencher™ 2 azide [TQ2 azide]                              | 5 mg     |
| 2231     | Tide Quencher™ 3 azide [TQ3 azide]                              | 5 mg     |
| 2068     | Tide Quencher™ 4WS azide [TQ4WS azide]                          | 1 mg     |
| 2070     | Tide Quencher™ 4WS-DBCO [TQ4WS-DBCO]                            | 1 mg     |
| 2088     | Tide Quencher™ 5.1WS azide [TQ5.1WS azide]                      | 1 mg     |
| 2082     | Tide Quencher™ 5WS azide [TQ5WS azide]                          | 1 mg     |
| 2097     | Tide Quencher™ 6WS azide [TQ6WS azide]                          | 1 mg     |
| 2120     | Tide Quencher™ 7.1WS azide [TQ7.1WS azide]                      | 1 mg     |
| 2128     | Tide Quencher™ 7.2WS azide [TQ7.2WS azide]                      | 1 mg     |
| 2112     | Tide Quencher™ 7WS azide [TQ7WS azide]                          | 1 mg     |
| 2136     | Tide Quencher™ 8WS azide [TQ8WS azide]                          | 1 mg     |
| 1446     | trFluor™ Eu DBCO *europium complex*                             | 100 µg   |
| 70002    | XFD350 azide  | 1 mg     |
| 70004    | XFD350 PEG4 DBCO  | 1 mg     |
| 70013    | XFD405 azide  | 1 mg     |
| 70015    | XFD405 PEG4 DBCO  | 1 mg     |
| 70024    | XFD430 azide  | 1 mg     |
| 70026    | XFD430 PEG4 DBCO  | 1 mg     |
| 1701     | XFD488 azide *Same Structure to Alexa Fluor™ 488 azide*         | 1 mg     |
| 1813     | XFD488 PEG4 DBCO  | 1 mg     |

| Cat. No. | Product          | Quantity |
|----------|------------------|----------|
| 70044    | XFD514 azide     | 1 mg     |
| 70046    | XFD514 PEG4 DBCO | 1 mg     |
| 1717     | XFD532 azide     | 1 mg     |
| 1719     | XFD532 PEG4 DBCO | 1 mg     |
| 70054    | XFD546 azide     | 1 mg     |
| 70056    | XFD546 PEG4 DBCO | 1 mg     |
| 1723     | XFD555 azide     | 1 mg     |
| 1725     | XFD555 PEG4 DBCO | 1 mg     |
| 70133    | XFD568 azide     | 1 mg     |
| 70135    | XFD568 PEG4 DBCO | 1 mg     |
| 1729     | XFD594 azide     | 1 mg     |
| 1731     | XFD594 PEG4 DBCO | 1 mg     |
| 70064    | XFD610 azide     | 1 mg     |
| 70066    | XFD610 PEG4 DBCO | 1 mg     |
| 70074    | XFD633 azide     | 1 mg     |
| 70076    | XFD633 PEG4 DBCO | 1 mg     |
| 70084    | XFD635 azide     | 1 mg     |
| 70086    | XFD635 PEG4 DBCO | 1 mg     |
| 1896     | XFD647 Azide     | 1 mg     |
| 1734     | XFD647 PEG4 DBCO | 1 mg     |
| 70094    | XFD660 azide     | 1 mg     |
| 70096    | XFD660 PEG4 DBCO | 1 mg     |
| 70104    | XFD680 azide     | 1 mg     |
| 70106    | XFD680 PEG4 DBCO | 1 mg     |
| 70114    | XFD700 azide     | 1 mg     |
| 70116    | XFD700 PEG4 DBCO | 1 mg     |
| 1738     | XFD750 azide     | 1 mg     |
| 1740     | XFD750 PEG4 DBCO | 1 mg     |
| 70123    | XFD790 azide     | 1 mg     |
| 70125    | XFD790 PEG4 DBCO | 1 mg     |

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