# 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., –$NH_2$, –SH, –COOH).
- **UV Traceability**: DBCO absorbs strongly at ~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.

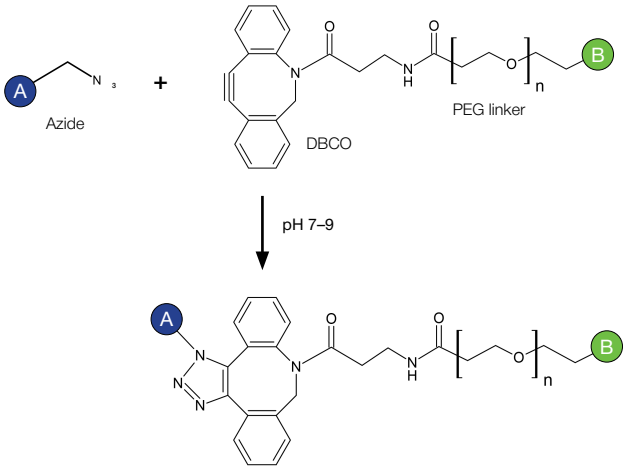


**Figure 1.** Schematic of SPAAC (Strain-Promoted Azide-Alkyne Cycloaddition) ligation reaction, in which a triazole bond is formed between an azide molecule and DBCO with high efficiency and specificity under mild conditions.

## 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 ~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 ~10 µL 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:

$$\mathrm{DBCO\,/Ab} = \frac{A_{309}\times\varepsilon_{\mathrm{Ab}}}{A_{280}-(\mathrm{CF}\times A_{309})\times\varepsilon_{\mathrm{DBCO}}}$$

Where:

- $\varepsilon_{DBCO}$ = 12,000 M−1 cm−1 at 309 nm
- $\varepsilon_{Ab}$ = 210,000 M−1 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

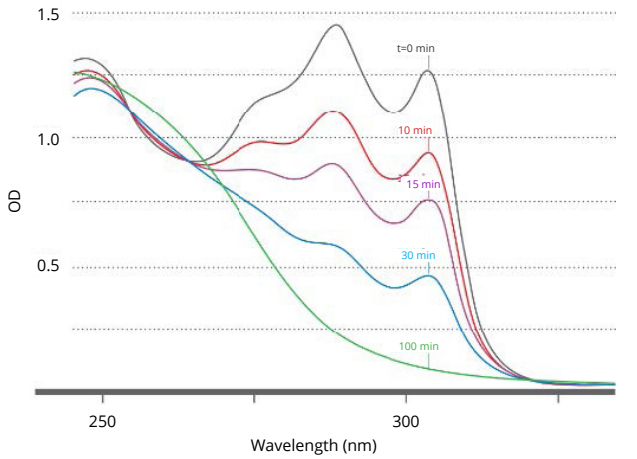


**Figure 2.** Real-time UV–Vis monitoring of the SPAAC (DBCO–Azide) reaction at various time points (0–100 min). The initial black spectrum (t=0 min) shows a pronounced DBCO peak near 310 nm, which progressively diminishes as the reaction proceeds. By 100 min (green line), the 310 nm peak is significantly 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 | ATTO 390 azide | 1 mg |
| 70205 | ATTO 390 DBCO | 1 mg |
| 70213 | ATTO 425 azide | 1 mg |
| 70215 | ATTO 425 DBCO | 1 mg |
| 2817 | ATTO 488 azide | 1 mg |
| 2819 | ATTO 488 DBCO | 1 mg |
| 2802 | ATTO 488 PEG3 azide | 1 mg |
| 70223 | ATTO 495 azide | 1 mg |
| 70225 | ATTO 495 DBCO | 1 mg |
| 2845 | ATTO 514 azide | 1 mg |
| 2847 | ATTO 514 PEG4 DBCO | 1 mg |
| 2824 | ATTO 532 PEG3 azide | 1 mg |
| 2821 | ATTO 532 PEG4 DBCO | 1 mg |
| 70233 | ATTO 550 azide | 1 mg |
| 70235 | ATTO 550 DBCO | 1 mg |
| 70243 | ATTO 590 azide | 1 mg |
| 70245 | ATTO 590 DBCO | 1 mg |
| 2840 | ATTO 594 PEG3 azide | 1 mg |
| 2842 | ATTO 594 PEG4 DBCO | 1 mg |
| 70253 | ATTO 610 azide | 1 mg |

**APPENDIX CONT.**

| Cat. No. | Product | Quantity |
|---|---|---|
| 70255 | ATTO 610 DBCO | 1 mg |
| 70263 | ATTO 620 azide | 1 mg |
| 70265 | ATTO 620 DBCO | 1 mg |
| 70273 | ATTO 633 azide | 1 mg |
| 70275 | ATTO 633 DBCO | 1 mg |
| 2832 | ATTO 647 PEG4 DBCO | 1 mg |
| 2835 | ATTO 647N azide | 1 mg |
| 2837 | ATTO 647N DBCO | 1 mg |
| 2806 | ATTO 647N PEG3 azide | 1 mg |
| 2807 | ATTO 647N PEG4 DBCO | 1 mg |
| 70283 | ATTO 655 PEG3 azide | 1 mg |
| 70285 | ATTO 655 PEG4 DBCO | 1 mg |
| 70293 | ATTO 680 PEG3 azide | 1 mg |
| 70295 | ATTO 680 PEG4 DBCO | 1 mg |
| 70303 | ATTO 700 PEG3 azide | 1 mg |
| 70305 | ATTO 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 |
| 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 |
| 1091 | iFluor® 647 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 |
| 24215 | MycoLight™ PMA [Propidium Monoazide] | 1 mg |
| 24216 | MycoLight™ PMA [Propidium Monoazide] *5 mM Water Solution* | 100 µL |
| 12605 | NTA Azide | 1 mg |

## APPENDIX CONT.

| Cat. No. | Product | Quantity |
|---|---|---|
| 5303 | Phalloidin-PEG4-DBCO | 100 µg |
| 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 |
| 50007 | Semicarbazide (SEM)-BSA Conjugate | 1 mg |
| 50009 | Semicarbazide (SEM)-HRP Conjugate | 1 mg |
| 50008 | Semicarbazide (SEM)-KLH Conjugate | 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] *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 |
| 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 |

## DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.