

Protocol for Click chemistry reactions with oYo-Link® DBCO

When working with oYo-Link DBCO (*Catalog #: AT3003*), you must perform the click chemistry coupling reactions with the Azide-labeled molecule of interest **first, prior to photo-crosslinking to an antibody**. UV illumination will damage the DBCO activity and thus, antibody conjugation should only be performed after the click-reaction. Also this is to avoid diluting oYo-Link DBCO prior to the reaction with the Azide-labeled molecule to maximize the reaction efficiency.

Copper-Free Click Chemistry Procedure:

- In PBS (pH 7.3), mix oYo-Link DBCO with a 1.5-fold molar excess of the Azide-labeled molecule of interest. The reaction should be kept at the highest concentration possible to maximize the reaction efficiency. Concentration is referenced on the Product Specification Sheet shipped with your product.
- Incubate for 2 hr at 37°C or incubate at 4°C overnight.
- Proceed to the antibody labeling protocol: <https://alphathera.com/user-manuals>

Notes:

- i** Azide-tagged molecules of interest include peptides, proteins and oligonucleotides. For peptides/ proteins, the linker length between Azide and the peptide/protein may affect the click reaction efficiency. For oligonucleotides, we recommend a C6 or PEG4 or longer linker between the Azide and the oligonucleotide.
- i** For some Azide-labeled molecules, longer reaction times may be required. In some cases, the reaction time can be up to 48 hours.
- i** Typically, no further purification is required prior to photo-crosslinking with an antibody. However, if purification is required, please follow the purification protocol of your choice, keeping in mind that oYo-Link has a molecular weight of ~8 kDa.

Storage & Stability Note:

- i** The dried product should be stored at -20°C upon receipt. Following reconstitution, DBCO loses its reactivity over time due to its oxidation and addition of water to the triple bond. The product should be stored at -20°C for immediate use (< 1-2 days) and -20°C to -80°C for longer time-spans. Freeze-thaw cycles should be limited to less than 10.

For additional questions please email support@alphathera.com