

Protocol for Click chemistry reactions with oYo-Link® Azide

When working with oYo-Link® Azide (Catalog #: AT3002), you must perform the click chemistry coupling reactions with the DBCO-labeled molecule of interest **first, prior to photo-crosslinking to an antibody**. UV illumination will damage the Azide activity and thus, antibody conjugation should only be performed after the click-reaction. Also this is to avoid diluting oYo-Link Azide prior to the reaction with the DBCO-labeled molecule to maximize the reaction efficiency.

- i** We do not recommend copper-catalyzed click chemistry reactions, because copper can affect oYo-Link photo-crosslinking efficiency

Copper-Free Click Chemistry Procedure:

- In PBS (pH 7.3), mix oYo-Link Azide with a 1.5-fold molar excess of the DBCO-labeled molecule of interest. The reaction should be kept at the highest concentration possible to maximize the reaction efficiency.
- 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** DBCO-tagged molecules of interest include peptides, proteins and oligonucleotides. For peptides/ proteins, the linker length between DBCO and the peptide/protein may affect the click reaction efficiency. For oligonucleotides, we recommend a C6 or PEG4 or longer linker between the DBCO and the oligonucleotide.
- i** For some DBCO-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.

