

Protocol for Click chemistry reactions with oYo-Link® Tetrazine

When working with oYo-Link® Tetrazine (Catalog #: AT3004), it is recommended to perform the click chemistry coupling reactions with a TCO (trans-Cyclooctene)-labeled molecule of interest first, prior to photo-crosslinking to an antibody. This is to avoid diluting oYo-Link Tetrazine prior to the reaction with the TCO-labeled molecule, to maximize the reaction efficiency.

Copper-Free Click Chemistry Procedure:

- In PBS (pH 7.3), mix oYo-Link Tetrazine with a 2.5-fold molar excess of the TCO-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** TCO-tagged molecules of interest include peptides, proteins and oligonucleotides. For peptides/proteins, the linker length between TCO and the peptide/protein may affect the click reaction efficiency.
- i** For some TCO-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.

