Protocol for Click chemistry reactions with oYo-Link® TCO

When working with oYo-Link® TCO (Catalog #: AT3005), it is recommended to perform the click chemistry coupling reactions with a Tetrazine-labeled molecule of interest first, prior to photo-crosslinking to an antibody. This is to avoid diluting oYo-Link TCO prior to the reaction with the Tetrazine-labeled molecule, to maximize the reaction efficiency.

Copper-Free Click Chemistry Procedure:

- PBS (pH 7.3), mix oYo-Link TCO with a 2.5-fold molar excess of the Tetrazine-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 **here**.

Notes:

Tetrazine-tagged molecules of interest include peptides, proteins and oligonucleotides. For peptides/proteins, the linker length between Tetrazine and the peptide/protein may affect the click reaction efficiency.

For some Tetrazine-labeled molecules, longer reaction times may be required. In some cases, the reaction time can be up to 48 hours.

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.