

## Protocol for Thiol-Maleimide reactions with oYo-Link® Thiol

When working with oYo-Link Thiol (Catalog #: AT3001), it is recommended that coupling reactions with a maleimide-labeled molecule of interest be performed first, prior to photo-crosslinking to an antibody. This is to avoid diluting oYo-Link Thiol prior to the reaction with the maleimide-labeled molecule, to maximize the reaction efficiency.

### Thiol-Maleimide Coupling Procedure:

- oYo-Link Thiol (33 uM concentration) is shipped in reduced form in H<sub>2</sub>O (pH4). Directly mix it with a 5-fold molar excess of the maleimide-labeled molecule of interest. The reaction should be kept at the highest concentration possible to maximize the reaction efficiency.
- Incubate for 2 hrs at RT or 37°C or incubate at 4°C overnight.
- Proceed to the antibody labeling protocol: <https://alphathera.com/user-manuals>

### Notes:

- **i** Maleimide-tagged molecules of interest include peptides, proteins and oligonucleotides. For peptides/ proteins, the linker length between Maleimide and the peptide/protein may affect the reaction efficiency.
- **i** For some cases, oYo-Link Thiol needs to be concentrated by spin filter (3kDa) before reaction to maleimide-labeled molecules.
- **i** For some Maleimide-labeled molecules, longer reaction times may be required.
- **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.

