We recommend using the following general protocol for Click chemistry labeling of alkyne-modified oligonucleotides with azides.

Click chemistry reaction takes place between two components: azide and alkyne (terminal acetylene). Both azido and alkyne groups are nearly never encountered in natural biomolecules. Hence, the reaction is highly bioorthogonal and specific. If there is a need to label an oligonucleotide, alkyne-modified oligonucleotides can be ordered at many of the custom oligo-synthesizing facilites and companies.

1. Calculate the volumes of reagents required for Click chemistry labeling using the table below. Prepare the required stock solutions (see **Appendix**).

Reagent	Final concentration in the mixture	Stock solution concentration
Oligonucleotide, alkyne-modified	Varies (20 – 200 uM)	varies
Azide	1.5 x (oligonucleotide concentration)	10 mM in DMSO
DMSO	50 vol %	-
Ascorbic acid	0.5 mM	5 mM in water
Cu-TBTA complex	0.5 mM	10 mM in 55 vol % DMSO

2. Dissolve **alkyne-modified oligonucleotide** or DNA in water in a pressure-tight vial.
3. Add **2M triethylammonium acetate buffer, pH 7.0**, to final concentration 0.2 M.
4. Add **DMSO**, and vortex.
5. Add **azide stock solution** (10 mM in DMSO), and vortex.
6. Add the required volume of **5mM Ascorbic Acid Stock solution** to the mixture, and vortex briefly.
7. Degass the solution by bubbling inert gas in it for 30 seconds. Nitrogen, argon, or helium can be used.
8. Add the required amount of **10 mM Copper (II)-TBTA Stock in 55% DMSO** to the mixture. Flush the vial with inert gas and close the cap.
9. Vortex the mixture thoroughly. If significant precipitation of azide is observed, heat the vial for 3 minutes at 80 ℃, and vortex.
10. Keep at room temperature overnight.

11. Precipitate the conjugate with acetone (for oligonucleotides) or with ethanol (for DNA). Add at least 4-fold volume of acetone to the mixture (If the volume of the mixture is large, split in several vials). Mix thoroughly and keep at -20 ℃ for 20 minutes.
12. Centrifuge at 10000 rpm for 10 minutes.
13. Discard the supernatant.
14. Wash the pellet with acetone (1 mL), centrifuge at 10000 rpm for 10 minutes.
15. Discard the supernatant, dry the pellet, and purify the conjugate by RP-HPLC or PAGE.

Appendix:

5 mM Ascorbic Acid Stock

Preparation	Dissolve 18 mg of ascorbic acid in 20 mL of distilled water.
Storage	Ascorbic acid is readily oxidized by air. The solution is stable for one day. Use fresh preparations for Click chemistry.

10 mM Copper (II)-TBTA Stock in 55% DMSO

Preparation	Dissolve 50 mg of copper (II) sulfate pentahydrate in 10 mL of distilled water. Dissolve 116 mg of TBTA ligand in 11 mL of DMSO. Mix two solutions.
Storage	Store at room temperature. The solution is stable for years.

2M Triethylammonium Acetate Buffer, pH 7.0

Preparation	Mix 2.78 mL of triethylamine with 1.14 mL of acetic acid. Add water to 10 mL volume, and adjust pH to 7.0.
Storage	Store at room temperature. The solution is stable for years.