

## **Protocol: Click-Chemistry Labeling of Biomolecules and DNA**

A variety of conjugates can be made through click chemistry reaction. Labeling DNA and other biomolecules is one of the applications of click chemistry. Biomolecules can be labeled with fluorescent dyes, biotin, and other labeling reagents.

Azide and alkyne are two key components for the click chemistry reaction. Because both azido and alkyne groups are nearly never encountered in natural biomolecules, the reaction is highly bioorthogonal and specific.

**The following protocol is recommended for the labeling of alkyne-modified biomolecules with azides.**

1. Calculate the volumes of reagents required for Click chemistry reaction.
2. Dissolve alkyne-modified biomolecule or DNA in water.
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. Bubbling inert gas (nitrogen, argon, or helium) in the solution for 30 seconds.
8. Add required amount of 10 mM Copper (II)-TBTA Stock in 55% DMSO to the mixture. Flush the vial with inert gas.
9. Vortex the mixture thoroughly. If precipitation of azide is observed, heat the vial for 3 minutes at 80°C, and vortex.
10. Keep at room temperature overnight.
11. The conjugate can be precipitated with either acetone (for oligonucleotides) or with ethanol (for DNA). Add at least 4-fold volume of acetone to the mixture. Mix thoroughly and keep at -20°C for 20 minutes.
12. Centrifuge at 10000 rpm for 10 minutes.
13. Discard the supernatant.
14. Wash the pellet with acetone, centrifuge at 10000 rpm for 10 minutes.
15. Discard the supernatant, dry the pellet, and purify the conjugate by RP-HPLC or PAGE.