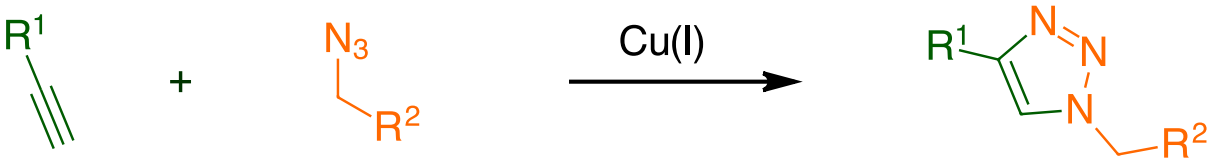
Conjugation of Alkyne-modified Oligonucleotides with Dye Azides

Click chemistry buffer is intended for post-synthetic conjugation of oligonucleotides containing alkyne. Oligonucleotides with terminal alkyne can be synthesized using alkyne phosphoramidite, or you can order synthesis of modified oligonucleotides on our website.

Azide is conjugated to terminal alkyne of a modified oligonucleotide, resulting in a five-membered heterocycle. Both groups (azide and alkyne) are extremely rarely found in natural biomolecules, so the reaction is highly specific and effective to handle various tasks.



The reaction proceeds in the presence of copper (I) at neutral pH. Catalytic buffer contains copper (II), triethylammonium acetate pH 7 and DMSO. It is recommended to use a freshly prepared solution of ascorbic acid to reduce copper (II).

For this reaction, you will need alkyne-modified oligonucleotide, dye azide, click chemistry buffer, and ascorbic acid. You can order all the reagents online on our website.

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Protocol

We recommend the following protocol for conjugation of alkyne-containing oligonucleotides with dye azides:

1. Determine total reaction volume based on the amount of oligonucleotide to be used:

Amount of oligonucleotide	Total reaction volume, µL
4 to 20 nmol	100
20 to 40 nmol	200
40 to 80 nmol	400
80 to 600 nmol	600

2. Calculate volumes of the reagents for the labeling reaction using the table below:

Reagent	Volume, µL	Concentration of stock solution
Dye azide	(amount of oligonucleotide [nmol]) × 0.15	10 mM in DMSO
Click chemistry buffer	(total reaction volume [µL]) × 0.67	1.5x
Activator (ascorbic acid)	(total reaction volume [µL]) × 0.02	50 mM in water
Water (for oligonucleotide dissolution)	(total reaction volume [µL] - volume of dye azide solution [µL] - volume of buffer [µL] - volume of activator solution [µL])	—

3. Prepare stock solution of *dye azide* (10 mM in DMSO) and *activator* (ascorbic acid, 50 mM in water).

   Bear in mind that ascorbic acid is readily oxidizable in air. Use only a freshly prepared solution of activator (the solution is stable within 1 day). To prepare stock solution, dissolve 10 mg of ascorbic acid in 1.1 mL of water.

4. Dissolve oligonucleotide in the calculated volume of *water* in a 2-mL plastic tube.
5. Add *click chemistry buffer* and vortex.
6. Add the calculated volume of stock solution of *dye azide* and vortex again.
7. *(recommended).* Degas the mixture to remove oxygen. To do so, connect a disposable pipette tip to a plastic or silicone tubing connected to the pressure regulator of a gas cylinder with inert gas (argon, nitrogen, or helium). Turn on very weak gas flow and put the tip down in the tube so that it can be 3–10 mm higher than the liquid level avoiding touching the liquid and tube walls. The gas flow should make a swirl in the liquid without spattering it. Keep the tip in this position for 10–20 seconds.

   If several labeling reactions are run simultaneously, a SpeedVac-type system can be used for degassing. To do so, place the tubes in the system, turn on rotation, turn on vacuum for 30–40 s, then turn off vacuum while feeding inert gas to the input of the system.
8. Add *activator solution (ascorbic acid)*, then purge the tube with inert gas for a few seconds and close it.
9. Vortex the solution. If a precipitate forms during the reaction, warm the tube up in hot water (70–95 °C) until the

precipitate dissolves and vortex the solution.

10. Allow the mixture to stand at room temperature for 8–16 h.
11. Add 2M solution of lithium perchlorate (1 volume per 5 volumes of the reaction mixture), vortex the solution, and add extra pure acetone to 2 mL.
12. Shake the tube and allow it to stand for 20 min at -20 °C.
13. Separate the precipitate by centrifuging at 10,000 RPM for 10 min. Discard the supernatant.
14. Add 1 mL of acetone to the precipitate. Shake the tube several times and separate the precipitate by centrifuging at 10,000 RPM for 10 min. Discard the supernatant.
15. Allow the precipitate to dry at room temperature in the open tube for 1 h or place the tube in the heating block at 65 °C for 10 min.
16. Dissolve the precipitate in water and purify the target product by HPLC.

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