Click Chemistry Oligonucleotide Labeling Protocol

1. Prepare the stock solutions of 2M $LiClO_4$, and sodium ascorbate (see Appendix).
2. Calculate the quantity OD_{260} units of acetylene-modified oligonucleotide. Use reagent volumes from Table 1.

Table 1. Quantities of reagents for labeling

OD_{260} units	**5<**	**5-10**	**10-15**	**15-20**	**20-25**	**25-30**	**30-35**	**35-40**	**40-45**	**45-50**
Oligonucleotide acetylene-modified, µL	30	60	90	120	150	180	210	240	270	300
2M TEAA (pH 7.0), µL	10	20	30	40	50	60	70	80	90	100
DMSO, µL	40	80	120	160	200	240	280	320	360	400
Click catalyst, µL	10	20	30	40	50	60	70	80	90	100
Sodium ascorbate (10 mM), µL	5	10	15	20	25	30	35	40	45	50

3. Calculate the volume of 10 mM dye-azide stock solution (**V**) using the equation:

$$V = 14\frac{OD_{260}n}{N}$$

where $OD_{260}units$ - quantity of acetylene-modified oligonucleotide in optical units
N - length of acetylene-modified oligonucleotide

V results in microliters.

4. Dissolve acetylene-modified oligonucleotide in water in a microcentrifuge tube.
5. Add 2M triethylammonium acetate buffer, pH 7.0.
6. Add DMSO, and vortex.
7. Add calculated volume of dye-azide stock solution (10 mM in DMSO), and vortex.
8. In a separate microcentrifuge tube mix required volumes of Click catalyst, sodium ascorbate, and vortex. Transfer the mixture to the microtube with oligonucleotide.
9. Degas the solution by bubbling inert gas for 1 minute and close the cap. Nitrogen or argon can be used.
10. Vortex the mixture thoroughly. If significant precipitation of dye-azide is observed, heat the tube for 3 minutes at 80 ºC, and vortex.
11. Keep at room temperature overnight.
12. Precipitate the conjugate with acetone: add 25 µL of 2M $LiClO_4$ per 100 µL of reaction mixture and at least 5-fold volume of acetone (if the volume of the mixture is large, split in several tubes). Mix thoroughly and keep at -20ºC for 30 minutes.
13. Centrifuge at 10.000 rpm for 10 minutes.
14. Discard the supernatant.
15. Wash the pellet with acetone (1 mL), centrifuge at 10.000 rpm for 10 minutes.
16. Discard the supernatant, dry the pellet, and purify the conjugate by RP-HPLC or PAGE.

Appendix. Preparation of stock solutions of the reagents used for click-chemistry labeling and conjugation

10 mM Sodium Ascorbate stock solution

Preparation: Dissolve sodium ascorbate (2 mg) in distilled water (1 mL).

Storage: Ascorbic acid is readily oxidized by air. The solution is stable for one day. Use fresh preparations for Click chemistry. Do not use yellowish solution.

2M $LiClO_4$ solution

Preparation: Dissolve 21.2 g of lithium perchlorate (or 32 g of lithium perchlorate trihydrate) in 100 mL of final volume of distilled water.

Storage: Store at room temperature. The solution is stable for years.