CuBr-based click reactions

This protocol was tested on DNA oligonucleotides, bearing a clickable alkyne. Aiming for bioconjugation with fluorescent labels in organic media, CuBr was used as catalytic reagent to promote the reaction. As CuBr is not very stable in air, often other catalysts are preferred. Anyhow, some reagents (e.g. biotins) and the need for an organic solvent media require this catalyst. We are therefore glad to share you our preferred protocol. Please be aware, that this protocol is only meant as a starting point. For other amounts and reaction partners used, please consider to vary the conditions in order to obtain the optimal reaction outcome.

You will need following reagents and equipment:

- CuBr (BCMI-001): the catalyst
- Click solution (BCMI-003) to dissolve the CuBr
- DMSO to dissolve the TBTA
- 100 mM solution of TBTA (BCMI-002) in DMSO to ligate the CuBr. This is important as the reagent is ensuring that no harmful oxidative species could be generated out of Cu.
- 10 mM solution of you preferred label-azide
- Eventually DMSO to dissolve your label-azide in case you bought is in solid form
- 100 µM solution of your alkyne-modified oligonucleotide
- Reaction tubes (e.g. 1.5 mL vials)
- Table centrifuge
- Thermomixer
- Purification (e.g. ethanol precipitation, purification kits, HPLC…)
- Analytical HPLC system

Click reaction procedure:

1. Dissolve the CuBr in click-solution (for each mg CuBr add 70 µL click-solution).
   Attention: please use this solution right away as it degrades! Observe the color of the solution: In case your solution turned brown and cloudy, please use a fresh CuBr.
2. Next, mix the CuBr-solution with the 100 mM solution of TBTA in a ration 1:2 to generate the „ready-to-use solution“using a table centrifuge. Also here: please use the mixture right after preparation.

3. 20 µL alkyne-oligonucleotide (100 µM) solution are then mixed (table centrifuge) with 5 µL „ready-to-use solution“ and 1 µL of label-azide (10 mM).
4. Let this mixture react for 3 hours at 37 °C and 600 rpm in a thermomixer.
5. Purify your dye-labeled oligonucleotide with your method of choice and elute / dissolve then your final oligo in HPLC grade water for further analyses and usages.
6. Check the quality of your probe e.g. by HPLC measurement.