CuBr-based click reactions

This protocol was tested on DNA oligonucleotides, bearing a clickable terminal 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 (e.g. Oligo² Labeling Kits). Anyhow, some reagents (e.g. biotins) and organic media require this compound. We are therefore glad to share with 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. Baseclick offers you various labels, custom oligos, click chemistry tools and purification kits. Please check our website for more information or get in touch with us.

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 (see our click chemistry tools section)
- Eventually DMSO to dissolve your label-azide in case you bought is in solid form
- 100µM solution of your alkyne-modified oligonucleotide (see custom oligo section)
- Reaction tubes (e.g. 1.5 mL vials)
- Table centrifuge
- Thermomixer
- Purification (e.g. ethanol precipitation, BaseClean kit, 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.
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 finally your oligo in HPLC grade water for further analyses and usage.
6. Check the quality of your probe e.g. by HPLC measurement.

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