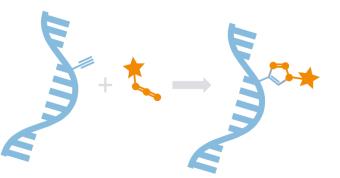


baseclick protocols

Cu-catalyzed click reactions (CuAAC) on mRNA

This protocol was tested on mRNA, bearing clickable alkyne. Aiming for bioconjugation with fluorescent labels CuSO₄ was used as catalytic reagent to promote the reaction. As CuAAC reactions require a Cu(I) source, a reducing agent is needed. The reaction conditions described below have been optimized to reduce Cu(II)-mediated degradation mRNA to a minimum. Please be aware, that this protocol is only meant



as a starting point and calculated for a final volume of 9 µL mRNA solution. For other amounts and reaction partners (e.g., tri-GalNAc BCFA-246 or other ligands and small tags), please consider specific optimization of reaction conditions.

Critical: do not include RNase inhibitor proteins in CuAAC reaction as the latter will be inhibited very efficiently.

You will need following reagents and equipment:

- A catalyst solution of 100 μ M CuSO₄ (BCMI-004) in RNase-free water
- 500 μM L-Ascorbic acid sodium salt (BCMI-005) in RNase free water for reduction of Cu(II) to the catalytically active Cu(I) state.
- A 200 μM solution of THPTA (BCMI-006) in RNase free water. THPTA is a Cu ligand used to increase click reaction rates and inhibit copper induced RNA damage
- alkyne-modified mRNA solution with a concentration of around 100 ng/ μ L or higher
- azide reagent in 10 times molar excess to alkyne-modified mRNA
- RNase free Microcentrifuge tubes (0.2-1.5 mL vials)
- Table-top centrifuge
- Heating block (e.g., Thermomixer or thermal cycler) or a water bath.
- Purification (e.g. spin column kits; see below)
- Gel electrophoresis equipment and Nanophotometer to verify integrity of the RNA and quantify recovery after purification.

Click reaction procedure (final volume of 9 µL):

- 1. Mix 1.5 μ l 100 μ M CuSO4 with 1.5 μ l 500 μ M sodium ascorbate. Incubate at least 1 min to reduce Cu(II) to Cu(I).
- 2. Add 1.5 μ I 200 μ M ligand (THPTA) and incubate at least 3 min to allow for Cu(I) chelation.
- 3. Add azide reagent in 10 times molar excess to alkyne-modified mRNA. Depending on the nature of the azide-containing tag lower molar ratio may be appropriate (this may require optimization).



baseclick protocols

page 2 of 2

- Add the RNA solution to the mixture. Use a RNA solution with a concentration of around 100 ng/μL or higher (higher concentrations result in higher click reaction rates). Adjust the total volume. Mix gently and spin down.
- 5. Incubate for 60 min at 45 °C.
- 6. After incubation add 1 μ l of 100 mM EDTA to quench the reaction.
- 7. Purify the clicked RNA. When clicking ligands with sugar moieties (e.g., Mannotriose BCFA-244 or tri-GalNAc), use spin column kits for DNA like the QIAquick PCR Purification Kit (Qiagen) or DNA Clean & Concentrator-10 (Zymo Research), otherwise the ethanolic binding buffers of RNA kits will lead to massive co-precipitation of excess ligand. With DNA spin-columns kits use a volume ration of Binding Buffer:sample = 7:1. If the amount of RNA is ≥ 7 µg either split the sample among as many columns as necessary to keep the loading ≤ 7 µg/column or use DNA Clean & Concentrator-25 or -100 kits. For other tags like fluorochromes or biotin spin column kits for purification of RNA are appropriate (e.g., RNA Clean & Concentrator-5, -25 from Zymo Research). Alternatively, precipitate RNA (should be ≥ 300 nt and ≥ 100 ng/µl) in the presence of LiCl to 2.5 M.