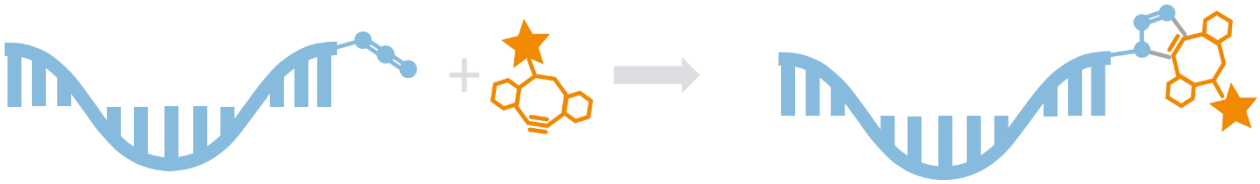
Strain-promoted click reactions (SPAAC) on mRNA

This protocol has been tested for conjugation of mRNA bearing an azide modification with dibenzocyclooctyne (DBCO)-modified fluorescent labels, targeting molecules like tri-GalNAc (BCFA-245) in aqueous solution. SPAAC (strain-promoted alkyne-azide cycloaddition) reactions are a popular alternative to conventional click chemistry involving a Cu(I) catalyst (CuAAC), especially for mRNA as they better preserve its integrity.



Please be aware, that this protocol is only meant as a starting point. For different amounts and reaction partners, please consider specific optimization of reaction conditions.

You will need following reagents and equipment:

- Nuclease-Free H_2O and 50 mM HEPES Buffer (pH = 7.2-7.5)
- around 0.02 nmol of azido -modified mRNA
- 2 nmol DBCO-modified coupling partner (label, dye, or biomolecule)
- RNase inhibitor 1 U/µL (inhibits CuAAC, but not SPAAC reactions)
- RNase free Microcentrifuge tubes (0.2- 1.5 mL vials)
- Table-top centrifuge
- 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 10-30 µL):

Below is our low scale protocol:

Reagent	Amount/concentration	Notes
HEPES buffer (pH 7.2-7,5)	Final 5 mM	RNase-free
(m)RNA	0.02 nmol	e.g., 10 µg of a 1500-1600 nt long RNA
DBCO-based reagent	2 nmol	100 equivalents
RNase inhibitor	1 U/µl	Optional: with clean handling not necessary
Total volume	10-30 µl	Keep volume as low as possible

1. Mix all reagents listed in the table.
2. The reaction mixture is incubated at room temperature overnight (~16 h; no needed for stirring).
3. Purify the solution with silica spin columns according to the user manual. When clicking ligands with sugar moieties (e.g., 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 coprecipitation 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 ≥ 300 nt and ≥ 100 ng/µl by adding LiCl to 2.5 M.

Additional operational notes:

- Aqueous stock solutions containing DBCO-modified molecules may not be stable for long-term use. Using DMSO as solvent usually extends their half-life and does not affect SPAAC reaction rates. Storage at –20 °C is recommended.
- The shelf-life of azido-modified nucleic acids do not differ from their unmodified counterparts.
- SPAAC-products become stable for long-term storage after completion of the click reaction.