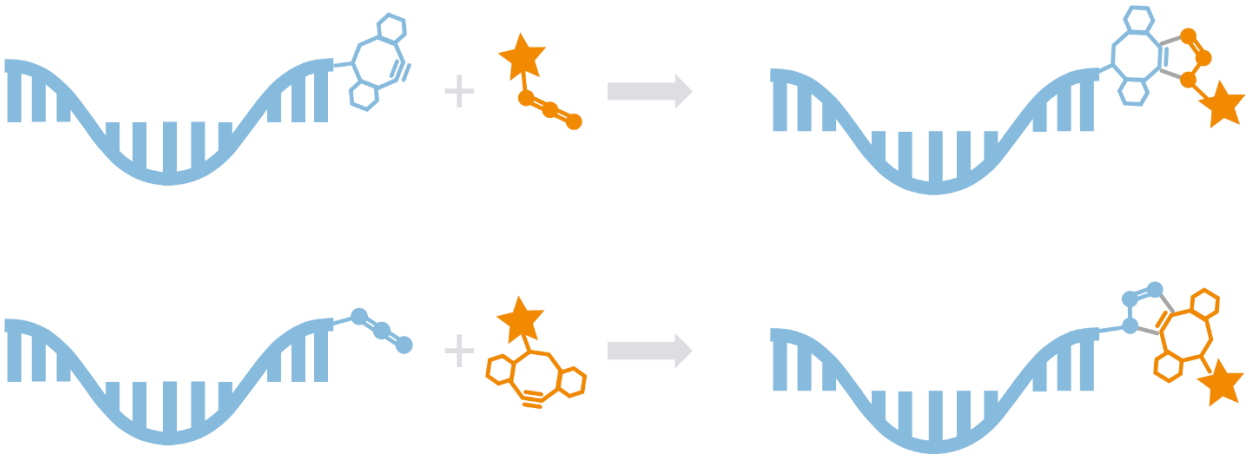
SPAAC- based click reaction

This protocol is suitable and has been tested for use with DNA and RNA oligonucleotides [1;2] bearing either azides or dibenzocyclooctyne (DBCO) modifications. Strain promoted alkyne-azide cycloaddition (SPAAC) reactions are a popular alternative to conventional click chemistry involving a Cu(I) catalyst.



Very similar to the Cu(I)-catalysed azide-alkyne cycloaddition (CuAAC), SPAAC reactions are biorthogonal and result in formation of a highly-stable 1,2,3-triazole linkage. Dyes, biotins, biomolecules and linkers are available for use in SPAAC chemistry either as azide or DBCO variant.
Therefore, we are glad to share our preferred reaction protocol for performing SPAAC reactions ourselves. 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.

Required Materials:

- DMSO and/or Nuclease-Free H_2O, and/or 5 mM HEPES Buffer (pH = 7.5)
- DBCO/azido-modified nucleic acid or oligonucleotide
- DBCO/azido-modified coupling partner (label, dye, or biomolecule)
- 1.5 mL Microcentrifuge tubes
- Heating Block / Mixer

Optional:

- Analytical HPLC system
- Benchtop Centrifuge
- Preferred spin column-based DNA/RNA purification system

Procedure:

1. Dissolve 1 mg of DBCO/azido-modified label, dye, biotin or biomolecule in DMSO or H_2O/HEPES buffer to obtain a 2 mM solution.
2. Dissolve or dilute an appropriate quantity of DBCO/azido-modified nucleic acid in H_2O/HEPES buffer to obtain a 0.4 mM solution.
3. Add an equal volume of the solution from **Step 1** to that of **Step 2**, centrifuge briefly, and incubate at room-temperature overnight (~16 h, no stirring is necessary).
4. Monitor the reaction progress using analytical RP-HPLC, where a successful reaction will be indicated by the appearance of two new peaks visible at λ = 260 nm while disappearance of the peak of your starting nucleic acid. Depending on the azide/alkyne used, the reaction will likely reach completion after 4-18 hours.
5. The reaction mixture can be purified using an appropriate spin column-based purification system.

Additional operational notes:

- Stock solutions containing DBCO-modified molecules may not be stable for long-term use. 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.

[1] Trimannose-coupled antimiR-21 for macrophage-targeted inhalation treatment of acute inflammatory lung damage: Nature Communications vol. 14, 4564 (2023)

[2] Targeted delivery to macrophages and dendritic cells by chemically modified mannose ligand-conjugated siRNA: Nucleic Acids Research Vol. 50, No. 9 (2022)