

Manual (05.05.2026)

Click Chemistry

General procedure and considerations

This manual contains some published examples of click reactions. These protocols may be used as a starting point for optimization of your particular click chemistry procedures. Please read the protocol carefully before starting.

For labeling you can use either non-fluorescent or fluorescent azides (e.g. Fluoresceine-, TAMRA-, Dabcyl- and Biotin-Azides). Please have a look at our [homepage](#), "Click Chemistry" section.

Preparation of DNA for Click

Custom synthesized oligos which are already alkyne-modified can be ordered from metabion.

You can also generate alkyne-modified DNA by PCR using alkyne-containing nucleotides (C8-Alkyne-dCTP, mi-N3001, C8-Alkyne-dUTP, mi-N3002) – please see our mi-Alkyne PCR Manual.

A. Click-Protocol for Oligonucleotide Labeling

Preparation of the "Oligonucleotide Solution"

Dissolve the oligonucleotide in the appropriate amount of water to adjust to 1 mM solution and centrifuge shortly. (Also different DNA-concentrations can be used!).

Preparation of the "Azide Solution"

- Warm up the azide to room temperature
- Add DMSO/t-Butanol Solvent to get a 10 mM solution
- Vortex the vial until the azide is dissolved completely
- Centrifuge shortly. The solution can be stored at -20 °C in the dark for several months. The azide functionality is very stable and does not hydrolyse in the presence of water.

Mix 10 µl of the "Oligonucleotide Solution" with 2 µl of the "Azide Solution".

Preparation of the "Click Solution"

The "click solution" must always be freshly prepared prior to use!

- Dissolve 54 mg TBTA in 1 ml DMSO/t-Butanol Solvent for a 0.1 M solution. This solution can be stored at -20 °C for several months.
- Dissolve 1 mg CuBr in 70 µl DMSO/t-Butanol Solvent to obtain a 0.1 M solution. This solution must be freshly prepared and cannot be stored.
- Add 1 volume of the 0.1 M CuBr solution quickly to 2 volumes of the 0.1 M TBTA solution to obtain "click solution", *ready-to-use*.

Click Procedure for Short DNA Oligos using CuBr

- Add 3 µl of the freshly prepared "Click Solution" to the previously mixed "Oligonucleotide/Azide Solution".
- The mixture should be thoroughly mixed and shaken at 40 °C for 4 h on a Thermomixer at 800 rpm. Alternatively shake the mixture over night at RT.
- The reaction is subsequently diluted with 0.3 M NaOAc (100 µl) and the DNA is precipitated using 1 ml cold EtOH.
- The supernatant is then removed and the residue is washed twice with 1 ml cold EtOH. The washed residue is re-dissolved in pure water (20 µl) and can be used without further purification.

B. Click-Protocol for Labeling a 300 bp PCR fragment

Preparation of the "DNA Solution"

Dissolve the DNA in the appropriate amount of water to adjust to 100 µM solution and centrifuge shortly. (Also different DNA-concentrations can be used!).

Preparation of the "Azide Solution"

See above

Mix 10 µl of the "DNA Solution" with 10 µl of the "Azide Solution".

Preparation of the "Click Solution"

See above

Click Procedure for a 300 bp PCR Product using CuBr

- Add 10 µl of the freshly prepared "Click Solution" to the previously mixed "DNA/ Azide Solution"
- The mixture should be thoroughly mixed and shaken at 40 °C for 4 h on a Thermomixer at 800 rpm. Alternatively shake the mixture over night at RT.
- The reaction is subsequently diluted with 0.3 M NaOAc (100 µl) and the DNA is precipitated using 1 ml cold EtOH.
- The supernatant is then removed and the residue is washed twice with 1 ml cold EtOH. The washed residue is re-dissolved in pure water (20 µl) and can be used without further purification.

C. General Considerations:

- The labeling reaction works more efficiently with concentrated solutions of alkynes (oligo) and azides (label).
- The best way to carry out the click reaction is to mix the oligo and the azide-label in a minimal amount of solvent.
- Alkyne / Azide ratio: from 1:2 to 1:10 for high density labeling reactions (e.g. 10 alkynes in a row).
- The click reaction is normally accelerated by elevated temperature and can be ready in less than 30 min when the reaction temperature is around 45 °C.
- The reaction time depends on: a) concentration of azide and oligo in the solution; b) reaction temperature; c) stirring and/ or mixing of the solution.
- The work-up of the reaction is normally carried out by precipitation of the labeled oligo (addition of a salt solution, e.g. 0.3 M NaOAc followed by addition of cold EtOH (100 %)).