Manual (19.09.2023)

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*.

metabion international AG | Semmelweisstrasse 3 | 82152 Planegg/Steinkirchen | Germany
phone: +49 89 899 363 0 | fax: +49 89 899 363 11 | email: info@metabion.com | www.metabion.com

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 %).