

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.

Preparation of the “Click Solution”

- The “click solution” (0.1 M CuBr / 0.1 M TBTA 1:2 in DMSO/t-BuOH 3:1) must always be freshly prepared prior to use!
- Dissolve 1 mg CuBr in 70 μ l DMSO/t-BuOH 3:1 to obtain a 0.1 M solution. This solution must be freshly prepared and cannot be stored.
- Dissolve 54 mg TBTA in 1 ml DMSO/t-BuOH 3:1 for a 0.1 M solution. This solution can be stored at -20 °C.
- 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

Procedure using CuBr: To 5 μ l of a 2 mM DNA solution (10 nmol) in water, 5 μ l of an azide solution (10 mM, 50 nmol, 5 eq.), 3 μ l of a freshly prepared solution containing 0.1 M CuBr and 0.1 M TBTA ligand in a 1:2 ratio in 3:1 DMSO/t-BuOH is added. The mixture is thoroughly mixed and shaken at 25 °C for 3-4 h. The reaction is subsequently diluted with 0.3 M NaOAc (100 μ l) 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 μ l) and can be used without further purification.

Considerations for the CuBr method:

- 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 highdensity 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 40 - 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.

Click Procedure using alternative Cu(I) Sources

Procedure using TCEP: To 25 μ l of a 0.5 mM DNA solution (12.5 nmol) in water, 6.25 μ l of an azide solution (0.1 N, 625 nmol) and 10 μ l of a solution containing Cu(II)-salt (CuSO₄) and TBTA ligand in a 1:2 ratio in 4:3:1 water/DMSO/t-BuOH is added (0.05 N, 250 nmol). The mixture is vortexed and 5 μ l of a freshly prepared tris-(2-carboxyethyl)-phosphine (TCEP) solution in water is added (0.1 N, 500 nmol). The solution is shaken at 15 °C over night and subsequently diluted with water (200 μ l) and used for gel electrophoresis without further purification. Instead of TCEP, also sodium-ascorbate can be used.

Click Procedure for a 300 bp PCR Product

To 10 μ l DNA solution (1-4 pmol DNA, 10 mM Tris), 10 μ l fluorescein azide solution (5 mM, diluted with 10 mM Tris with 5 % t-BuOH from a stock of 0.1 N in DMSO) and 10 μ l pre-complexed Cu(I) was added (10 mM; 1 mg CuBr (99.99%) dissolved in 700 μ l of 10 mM TBTA ligand in t-BuOH/DMSO 1:3) The sample is shaken at 37 °C for 2 h. Then formamide buffer is added and the samples are analyzed using a 5 % PAGE gel. Control experiments show that the reaction is completed in less than 30 min.