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Click Chemistry Protocols

Classic Click Chemistry uses copper, Cu(I), to catalyze the 1,3-dipolar cycloaddition of an alkyne with an azide to form a 1,2,3-triazole.[1,2] Sources of Cu(I) include copper(I) iodide, copper(I) bromide, copper turnings, or copper(II) sulfate ($CuSO_4$) and a reducing agent.[1] However, the thermodynamic instability of Cu(I), which readily oxidizes to inactive Cu (II) usually requires the copper catalyst to be prepared with an appropriate chelating ligand.

An improvement in Click Chemistry uses the in situ preparation of Cu(I) from the reduction of $CuSO_4$ with sodium ascorbate and a Cu(I) stabilizing ligand, tris-(benzyltriazolylmethyl)amine (TBTA).[3] This leads to a more reliable click reaction by avoiding the oxidation of catalytic Cu(I) by dissolved oxygen. In a typical reaction, copper sulfate is pre-complexed with TBTA. This complexed catalyst is mixed with an alkyne and an azide, followed by the addition of sodium ascorbate to initiate the click reaction.

TBTA covers some of the practical applications for Click Chemistry except for aqueous conjugation reactions. The water-soluble tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) click ligand further simplifies Click Chemistry by allowing the entire reaction to be run in water, affording biological compatibility for Click reactions.[4] The THPTA ligand binds Cu(I), blocking the bioavailability of Cu(I) and ameliorating the potential toxic effects while maintaining the catalytic effectiveness in click conjugations. The THPTA ligand was effectively used to label live cells with high efficiency while maintaining cell viability.[5]

In our hands, we have found THPTA to be a highly efficient ligand for click chemistry, in partially in completely aqueous reactions. Labeling is complete in as little as 15-30 minutes at room temperature. The ligand $CuSO_4$ complex exhibits no loss of activity when frozen for at least a month.

Example Protocols:

This section contains some general protocols for click reactions. These protocols may be used as a starting point for optimization of your particular click chemistry procedures. The auxiliary reagents and complete kits can be ordered from Click Chemistry Tools.

Labeling of Oligonucleotides and DNA

1. Prepare the following stock solutions:
   - 200 mM THPTA ligand in water
   - 100 mM CuSO4 in water
   - alkyne labeled oligo in water
   - 100 mM sodium ascorbate in water
   - 10 mM azide in DMSO/tBuOH or water.
2. Incubate $CuSO_4$ with THPTA ligand in a 1:2 ratio several minutes before the reaction. This solution is stable for several weeks when frozen.
3. To the oligo/DNA solution, add an excess of azide (4-50 eq).
4. Add 25 equivalents of THPTA/$CuSO_4$.
5. Add 40 equivalents of sodium ascorbate.
6. Let the reaction stand at room temperature for 30-60 minutes.
7. Ethanol-precipitate the oligo or purify.

Labeling of Cell Lysate

1. Prepare the following click solutions:
   - 100 mM THPTA ligand in aqueous buffer or water
   - 20 mM CuSO4 in water
   - 300 mM sodium ascorbate in water
   - 2.5 mM alkyne or azide labeling reagent in water or DMSO
2. For each azide or alkyne- modified protein lysate sample, add the following to a 1.5 mL microfuge tube, then vortex briefly to mix.
   - 50 µL protein lysate (1-5 mg/mL) in protein extraction buffer
   - 90 µL PBS buffer
   - 20 µL of 2.5 mM corresponding azide (or alkyne) detection reagent in DMSO or water
3. Add 10 µL of 100 mM THPTA solution, vortex briefly to mix.
4. Add 10 µL of 20 mM $CuSO_4$ solution, vortex briefly to mix.
5. Add 10 µL of 300 mM sodium ascorbate solution to initiate click reaction, vortex briefly to mix.
6. Protect reaction from light and allow click reaction to incubate for 30 minutes at room temperature.
7. Proteins in lysate are now click labeled and ready for downstream processing and/or analysis.

Preparation of Samples for Gel Analysis

1. Add 600 µL methanol to 200 µL reaction mixture, vortex briefly.
2. Add 150 µL chloroform, vortex briefly.
3. Add 400 µL dH_2O, vortex briefly.
4. Centrifuge for 5 minutes at 13,000-20,000 x g, carefully remove upper aqueous layer without disturbing interface layer containing proteins. Note-upper aqueous layer may either be colorless or contain color depending on detection reagent.
5. Add 450 µL methanol, vortex briefly.
6. Centrifuge for 5 minutes at 13,000-20,000 x g to pellet protein. Carefully, remove and discard supernatant.
7. Add 450 µL methanol, vortex briefly. Repeat step 6.
8. Open the lid to microfuge tube and allow protein pellet to air-dry for at least 15 minutes.
9. Cap and store labeled sample at -20^{0}C until ready for use.

Labeling of Live Cells

See Hong, V., et al (ref. 5) for detailed protocol and suggestions.

References

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5. Hong, V., et al (2010). Labeling Live Cells by Copper-Catalyzed Alkyne–Azide Click Chemistry. *Bioconjugate Chem.*, **21(10)**, 1912-1916.