Labeling Azide-Modified Biomolecules with Fluorescent Dye Alkynes

Labeling Oligonucleotides with Dye Alkynes

1. Prepare the following stock solutions:
   - 200 mM THPTA [tris(3-hydroxypropyltriazolylmethyl)amine)] in water
   - 100 mM CuSO4 in water
   - Azide-modified oligo in water (as concentrated as possible, e.g., >10 mg/ml)
   - 100 mM sodium ascorbate in water
   - 10 mM dye alkyne in DMSO or water (see our website for recommended solvent)
2. Mix and vortex well CuSO4 with THPTA in a 1:2 ratio for several minutes before the reaction. This working solution is stable for several weeks when frozen.
3. To the azide-modified oligo solution, add an excess of dye alkyne (2-5 equivalents by molar ratio).
4. Add 5 equivalents of THPTA/CuSO4 working solution (from Step 1)
5. Add 10-30 equivalents of sodium ascorbate.
6. Stir, vortex or shake the reaction mixture at room temperature for 30-60 minutes.
7. Ethanol-precipitate the oligo or purify it by your desired method (e.g., HPLC).

Labeling Peptides with Dye Alkynes

1. Prepare the following stock solutions:
   - 200 mM THPTA ligand in water
   - 100 mM CuSO4 in water
   - Azide-modified peptide in water or DMF (depending on your peptide solubility, >10 mg/ml if possible)
   - 100 mM sodium ascorbate in water
   - 10 mM dye alkyne in DMSO or water (see our website for recommended solvent)
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 azide-modified peptide solution, add an excess of dye alkyne (5-10 equivalents by molar ratio).
4. Add 5-10 equivalents of THPTA/$CuSO_4$.
5. Add 10-20 equivalents of sodium ascorbate.
6. Stir, vortex or shake the reaction mixture at room temperature for 30-60 minutes.
7. Purify your desired peptide by HPLC.

Labeling Small Organic Alkyne Molecules with Dye Alkynes

1. Prepare the following stock solutions:
   - 200 mM THPTA ligand in water
   - 100 mM CuSO4 in water
   - Alkyne compound in water or DMF (depending on your compound solubility, >10 mg/ml if possible,)
   - 100 mM sodium ascorbate in water
   - 10 mM dye alkyne in DMSO or water (see our website for recommended solvent).
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 alkyne solution, add an excess of dye alkyne (5-10 equivalents by molar ratio).
4. Add 25 equivalents of THPTA/$CuSO_4$.
5. Add 50 equivalents of sodium ascorbate.
6. Stir the reaction mixture at room temperature for 30-60 minutes.
7. Purify your desired molecule by chromatography or other methods.

Labeling Biopolymers with Dye Alkynes

1. Prepare the following stock solutions:
   - 200 mM THPTA ligand in water
   - 100 mM CuSO4 in water
   - Azide-modified biopolymer in water (as concentrated as possible, e.g., >5 mg/ml)
   - 100 mM sodium ascorbate in water
   - 10 mM dye alkyne in DMSO or water (see our website for recommended solvent).
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 azide-modified biopolymer solution, add an excess of dye alkyne (Loading ratio: 5-20 dye alkyne/alkyne).
4. Add 5 molar equivalents (referenced to dye alkyne) of THPTA/$CuSO_4$.
5. Add 10 equivalents of sodium ascorbate (referenced to dye alkyne).
6. Stir, vortex or shake the reaction mixture at room temperature for 30-60 minutes.
7. Purify your desired molecule by gel filtration or dialysis.

Labeling Cells, Cell Lysates or Biological Samples with Dye alkynes or Dye Alkynes

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 azide-modified cell or cell lysate sample, add the following reagents to a 1.5 mL microfuge tube, then vortex briefly to mix.
   - 50 µL cell or cell lysate sample
   - 50 µL PBS buffer
   - 50 µL of 5 mM corresponding dye alkyne (or dye 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 CuSO4 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. Cells or cell lysates are now click labeled and ready for downstream processing and/or analysis.