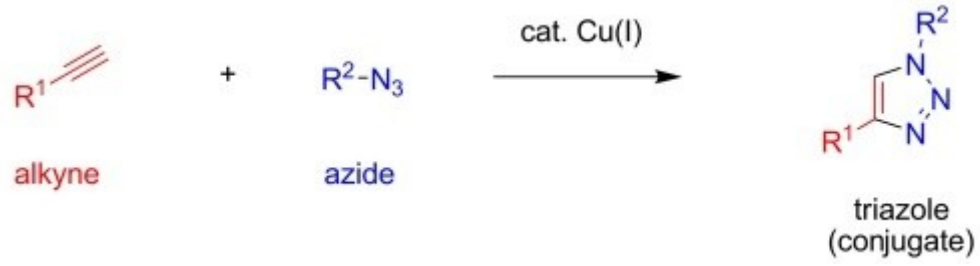
Product data sheet
Interchim Innovations
Interbiotech - BioScience Innovations

Click Chemistry: new protocol for the labeling and modification of biomolecules

Click Chemistry is a reaction between azide and alkyne yielding covalent product - 1,5-disubstituted 1,2,3-triazole. This process is also known as CuAAC - Cu catalyzed alkyne azide cycloaddition.



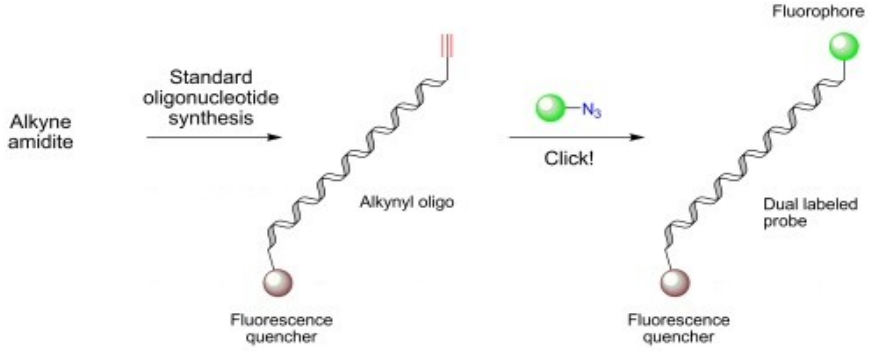
Click Chemistry is based on copper catalysis. The catalyst is often introduced as Cu-TBTA complex.

Among the vast variety of organic reactions, Click Chemistry has been selected as a conjugation chemistry reaction because of several advantages.

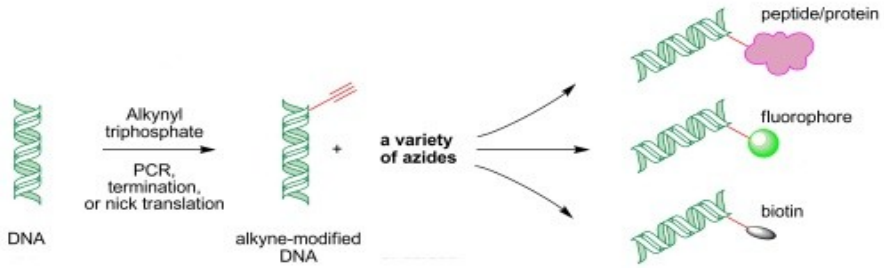
- **It is very selective.** Click Chemistry reaction takes place only between azide and alkyne components. It is does not interfere with most any other organic groups present in DNA and proteins being labeled, such as amino and carboxy groups.
- **There are no azides and alkynes in native biomolecules.** These groups should be specially introduced into DNA and proteins. Alkyne-containing DNA can be prepared with alkyne phosphoramidite during standard oligo synthesis. Proteins labeled with azide and alkyne can be made using azide activated ester and alkyne activated ester.
- **Click Chemistry takes place in water.** Aqueous DMSO, DMF, acetonitrile, alcohols, or pure water and buffers can be used for the reaction. The reaction is biocompatible and can take place in living cells.
- **Reaction is quick and quantitative.** Click Chemistry is a tool that allows preparation of nanomols of conjugates in diluted solutions.
- **The reaction is pH-insensitive.** Unlike reaction of NHS esters with amines, and some other conjugation chemistries, there is no need to control pH in reaction mixture. There is no need to add any special buffer, acid or base - Click Chemistry works well in pH interval of 4-11.
- **Protocol is simple!** For example see our recommended DNA labeling protocol.

Click Chemistry thus became a tool for universal modification of DNA, proteins, conjugate preparation, and fluorescent labeling. This is where Lumiprobe comes to help - we provide reagents and protocols for the facile and efficient synthesis of diverse azido- and alkyne-labeled biomolecules, as well as reactive fluorescent dyes and other reporter groups. With these reagents, you can perform easy preparation of conjugates in your lab. Here are just several examples.

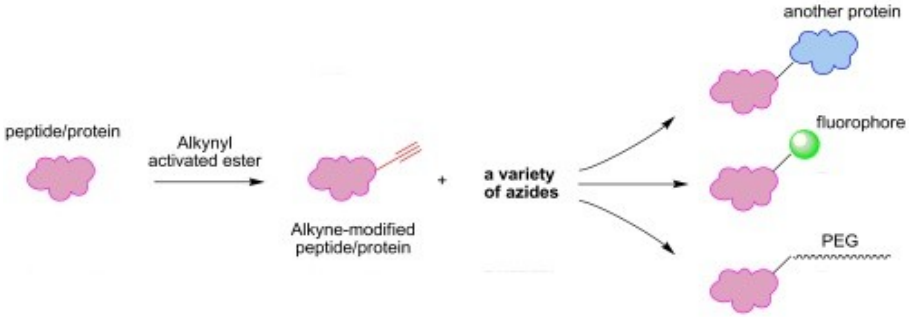
- **Fluorescent labeled oligonucleotides & dual-labeled probes for realtime PCR.** We provide alkyne phosphoramidites for easy synthesis of alkyne modified oligos, and fluorescent dye azides. Based on Click Chemistry, this technology provides significant advantages over labeling via activated esters or fluorescent dye amidites.



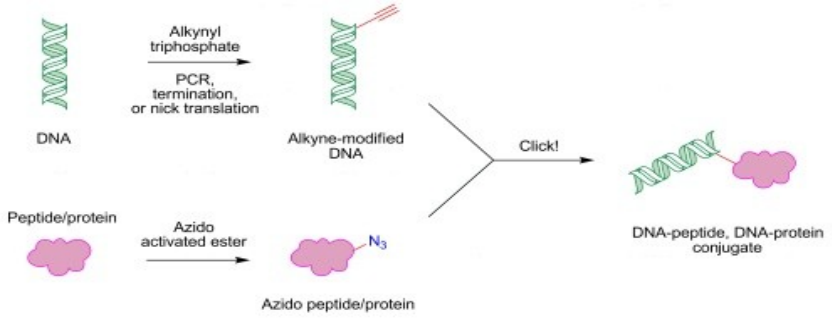
- **Fluorescent & biotinylated DNA.** Use alkynyl triphosphates for the incorporation of alkyne in DNA by PCR, termination, or nick translation. You can thereafter label DNA with any dye or biotin in your lab, without need in specific labeled triphosphates!



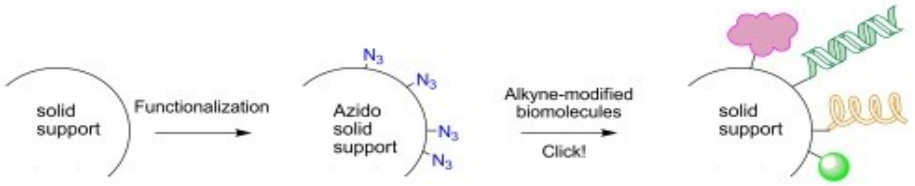
- **Fluorescent peptides, proteins, and antibodies.** We provide alkyne and azide activated esters for the modification of proteins and peptides with either azide or alkyne. You can use alkyne- or azido-modified proteins for the preparation of conjugates with DNA, reporter groups and solid surfaces.



- **Peptide-oligonucleotide conjugates.** We provide azido activated ester for the labeling of peptides, and alkyne amidites for the synthesis of alkyno oligos.



- **Biomolecules immobilized on nearly any solid phase.** We would be glad to consult you on the modification of solid surfaces, provide you with custom solid phases, and guide you to success!



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Protocol: Click-Chemistry Labeling of Oligonucleotides and DNA

Click chemistry is a versatile reaction that can be used for the synthesis of a variety of conjugates. Virtually any biomolecules can be involved, and labeling with small molecules, such as fluorescent dyes, biotin, and other groups can be readily achieved.

Click chemistry reaction takes place between two components: **azide** and **alkyne (terminal acetylene)**. Both azido and alkyne groups are nearly never encountered in natural biomolecules. Hence, the reaction is highly bioorthogonal and specific. If there is a need to label an **oligonucleotide**, alkyne-modified oligonucleotides can be ordered at many of the custom oligo-synthesizing facilites and companies.

We recommend using the following general protocol for Click chemistry labeling of alkyne-modified oligonucleotides with azides produced by Lumiprobe LLC. The auxiliary reagents can be ordered at Lumiprobe LLC.

1. Calculate the volumes of reagents required for Click chemistry labeling using the table below. Prepare the required stock solutions (see Appendix).

Reagent	Final concentration in the mixture	Stock solution concentration
Oligonucleotide, alkyne-modified	Varies (20 – 200 uM)	varies
Azide	1.5 x (oligonucleotide concentration)	10 mM in DMSO
DMSO	50 vol %	-
Ascorbic acid	0.5 mM	5 mM in water
Cu-TBTA complex	0.5 mM	10 mM in 55 vol % DMSO

2. Dissolve **alkyne-modified oligonucleotide** or DNA in water in a pressure-tight vial.
3. Add **2M triethylammonium acetate buffer, pH 7.0**.
4. Add **DMSO**, and vortex.
5. Add **azide stock solution** (10 mM in DMSO), and vortex.
6. Add the required volume of **5mM Ascorbic Acid Stock solution** to the mixture, and vortex briefly.
7. Degass the solution by bubbling inert gas in it for 30 seconds. Nitrogen, argon, or helium can be used.
8. Add the required amount of **10 mM Copper (II)-TBTA Stock in 55% DMSO** to the mixture. Flush the vial with inert gas and close the cap.
9. Vortex the mixture thoroughly. If significant precipitation of azide is observed, heat the vial for 3 minutes at 80°C, and vortex.
10. Keep at room temperature overnight.
11. Precipitate the conjugate with acetone (for oligonucleotides) or with ethanol (for DNA). Add at least 4-fold volume of acetone to the mixture (If the volume of the mixture is large, split in several vials). Mix thoroughly and keep at -20°C for 20 minutes.
12. Centrifuge at 10000 rpm for 10 minutes.
13. Discard the supernatant.
14. Wash the pellet with acetone (1 mL), centrifuge at 10000 rpm for 10 minutes.
15. Discard the supernatant, dry the pellet, and purify the conjugate by RP-HPLC or PAGE.

Appendix. Preparation of stock solutions of the reagents used for click-chemistry labeling and conjugation

5 mM Ascorbic Acid Stock

Preparation: Dissolve 18 mg of ascorbic acid in 20 mL of distilled water.

Storage: Ascorbic acid is readily oxidized by air. The solution is stable for one day. Use fresh preparations for Click chemistry.

10 mM Copper (II)-TBTA Stock in 55% DMSO

Preparation: Dissolve 25 mg of copper (II) sulfate pentahydrate in 10 mL of distilled water. Dissolve 58 mg of TBTA ligand in 11 mL of DMSO. Mix two solutions.

Storage: Store at room temperature. The solution is stable for years.

2M Triethylammonium Acetate Buffer, pH 7.0

Preparation: mix 2.78 mL of triethylamine with 1.14 mL of acetic acid. Add water to 10 mL volume, and adjust pH to 7.0.

Storage: Store at room temperature. The solution is stable for years.

Products

• EdU Cell Proliferation Assay	FP-MM982A	• Cy5 azide	FP-EV0910
• FluoProbes 488 azide	FP-YE4970	• FAM azide, 5-isomer	FP-EV0920
• FluoProbes 532A azide	FP-YE4980	• FAM azide, 6-isomer	FP-EV0930
• FluoProbes 550A azide	FP-FI2090	• JOE azide, 5-isomer	FP-EV0940
• FluoProbes 565A azide	FP-YE4990	• ROX azide, 5-isomer	FP-EV0950
• FluoProbes 590A azide	FP-YE5000	• ROX azide, 6-isomer	FP-EV0960
• FluoProbes 633A azide	FP-YE5010	• TAMRA azide, 5-isomer	FP-EV0880
• FluoProbes 647N azide	FP-YE5020	• DMSO anhydraous	FP-JW7390
• FluoProbes 655A azide	FP-YE5030	• CuSO4 5H2O	13495A
• Cy3 azide	FP-EV0900	• PBS powder	68723A

Product information sheet

FluoProbes azide	data sheet
EdU Cell Proliferation Assay	data sheet