FluoProbes AF Alkyne

*Alkynes **AF** dyes for fluorescent labeling of biomolecules with azide groups via a copper-catalyzed click reaction (CuAAC)*

Introduction

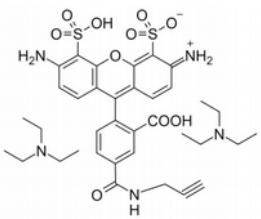
A variety of **AF** dyes has been used to label proteins, nucleic acids and other biomolecules for fluorescence techniques (imaging, biochemical analysis). They replace advantageously the conventional fluorochromes such as Fluorescein (FITC) and rhodamines (TRITC, RRX). The dyes are water soluble and pH-insensitive from pH 4 to 10.

AF350: It is a blue-fluorescent dye optimal for use with the 350 nm UV laser.
AF488: It is a bright green-fluorescent dye optimal for use with the 488 nm Argon laser.
AF594: It is a bright red-fluorescent dye optimal for use with the frequency-doubled He-Ne laser line.

FluoProbes® **AF** Alkynes are reactive dyes for the labeling of azide-groups via a copper-catalyzed click reaction (CuAAC). This click chemistry is increasingly being used in a variety of biological applications. They are reactive with terminal alkynes via a copper-catalyzed click reaction as a bio-orthogonal or biologically unique hapten for use in applications requiring signal amplification.

Products Description

The table below gives main physical and fluorescence characteristics of the activated dyes.

Product name cat.number/qty	**MW** g·mol^{-1} **(+added MW)**	**λ abs./em.** nm	**mol. abs.** M^{-1}cm^{-1}	Comment, structure
AF350 – Alkyne On demand, 1mg Soluble in DMSO		346 / 442	19 000	
AF488 – Alkyne FP-AXCECA, 1mg Soluble in DMSO	773.92	494 / 517	76 000 QY: 0.92	
AF594 – Alkyne On demand, 1mg Soluble in DMSO		590 / 617	92 000 QY: 0.66	

Storage: –20°C, protected from light (+4°C possible for short term) (M)
Avoid prolonged exposure to light. Desiccate.
Stable for 12 months after receival at -20°C in the dark. Transportation: at room temperature for up to 3 weeks.

Technical and Scientific Information

Click Chemistry

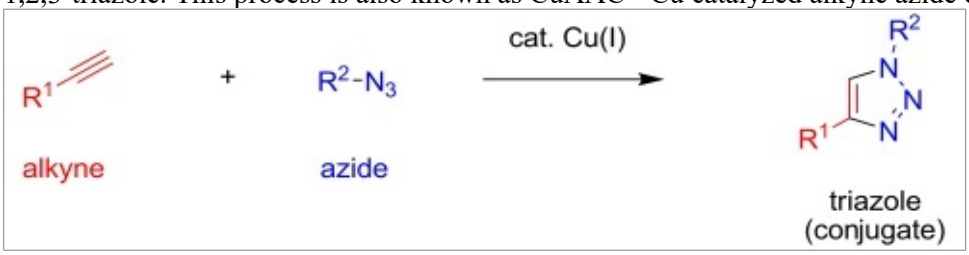
Among the vast variety of organic reactions, Click Chemistry has been selected as a conjugation chemistry reaction because of several advantages:

►**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 nanomoles 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!** See below.

Click Chemistry thus became a tool for universal modification of DNA, proteins, conjugate preparation, and fluorescent labeling. This is where our reagents come to help: you can perform easy preparation of conjugates in your lab.

- **Click Chemistry reaction – CuAAC**

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.



The catalyst is often introduced as Cu-TBTA (**Copper(II)-TBTA complex** FY2780).

Protocols

Following are directions for (A) use for incorporating the alkyne and the azide moieties into partner molecules to be conjugated, and for (B) a standard protocol for performing the click coupling reaction between these derivatized partners.

Guidelines A(r) : Biomolecules derivatization for Click chemistry

Partner molecules to be conjugated by Click reaction (according protocol B) should be derivatized to contain respectively an azide group and an alkyne group.

For amine containing molecules this can be easily achieved using the Azide Activator (ZL5540) and Alkyne Activator (ZL5530) reagents. Both reagents react with free amines in aqueous solutions at pH 7.5-9 (also react in organic solvents).
Please refer to protocols provided for acylating reagents such as NHS-biotin #R2027A.

FT-AXCECA

Furthermore,

- the azide and alkyne groups can be incorporated in peptide or oligonucleotides sequence during solid phase synthesis (see alkyne and azide building blocks in related products).
- alkyne- or azide- modified oligonucleotides or peptides can be ordered on custom synthesis (please inquire)
- finally, several labels are available already derivatized with azide (and also with alkyne) -see related products -.

Protocol B(r) : Click Chemistry Labeling of Oligonucleotides and DNA

We recommend using the following general protocol for Click chemistry labeling of alkyne-modified oligonucleotides with azides containing molecules such as biotin of Fluorescent labels. See related products for the auxiliary reagents.

Note: The protocol may be adapted for peptides, proteins and any other molecules including alkyne groups.

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 µM)	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 in water in a pressure-tight vial.
3. Add 2M triethylammonium acetate buffer, pH 7.0.
4. Add DMSO, and vortex.
5. Add azide containing molecule 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. Degas 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 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 80ºC, and vortex.
10. Keep at room temperature overnight.
11. Desalt by suitable method (Dialysis, Ultrafiltration, Precipitation – see related products).

Following is a procedure for desalting and concentration by precipitation:

- Precipitate the conjugate with acetone. 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 ºC for 20 minutes
- Centrifuge at 10000 rpm for 10 minutes.
-Discard the supernatant.
-Wash the pellet with acetone (1 mL), centrifuge at 10000 rpm for 10 minutes.
-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: ready to use product #FY2780

2M Triethylammonium Acetate Buffer, pH 7.0

FT-AXCECA
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.

Related products

* **CY$_{anine}$ dyes** functionalized by **NHS** (BB7493), **Maleimide** (JO6660), **Azide** (HO7250), **Alkyne** (1A6320), **Hydrazide** (LQV050), **DBCO** (DQP790: CycloAlkynes, for strain-promoted Click reactions), **Amino** group (CY3AM0), **Carboxyl** group (CY3CA0). 2D DI GE **3Dye labeling kit** (CY$_{anine}$2/CY$_{anine}$3/CY$_{anine}$5) (EV0870)

* Superior **FluoProbes fluorescent dyes**
- activated by –NHS (list), i.e. FP488-NHS #BA6800
- activated by –Maleimide (list), i.e. FP488-MAL #BA6810
- activated by –Azide, i.e. FP488-Azide #YE4970

* Classic dyes such as **FAM, R110, JOE TAMRA,** and **ROX.**

* Fluorescently labeled ligands:
• **Labeled lectins**, i.e. ConA-CY$_{anine}$3 #FT-WT868.
• **Labeled secondary antibodies**
• **Labeled tags**, i.e. CY$_{anine}$3-polylysine #FT-WT8550

* Other labeling/conjugation chemistries: • Click Chemistry reagents

Ordering information

Catalog size quantities and prices may be found at www.interchim.com/
Please inquire for higher quantities (availability, shipment conditions).
For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06