

Click chemistry Activators

Convenient activation of amine containing biomolecules for Click Chemistry conjugations
i.e. conjugation of Alkyne and Azido functional groups to proteins, peptides, aminoallyl-modified oligos.

Products Description

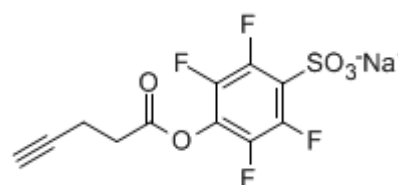
Name : **Alkyne Amine-Activator**

Pentynoic acid ester with amine reactivity

Catalog Number : ZL5530, 5mg ZL5531, 25mg ZL5532, 100mg

Molecular Weight : MW= 348.20

Added mass after coupling:



Activated ester

reagent for the **alkyne labeling** of amino-containing biomolecules.

The reagent is water-soluble.

After modification with this reagent, alkyne-modified biomolecules can be labeled with various azido-dyes via Click Chemistry reaction.

Properties: Amine reactive

Storage Store frozen ^(M)

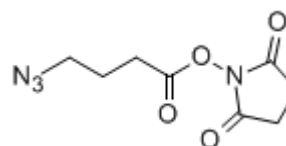
Name : **Azide Amine-Activator**

Azidobutyric acid ester with amine reactivity

Catalog Number : ZL5540, 5mg ZL5541, 25mg ZL5542, 50mg

Molecular Weight : MW= 226.19

Added mass after coupling: 127.1



Activated ester reagent for the **azido-labeling** of amino-containing biomolecules.

After modification with this reagent, azido-biomolecule can be reacted with various alkyne-containing compounds, such as alkyno-oligonucleotides.

Properties: Amine reactive

Storage Store frozen ^(M)

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Introduction

Click chemistry is a versatile reaction that takes place between two groups: **azide** and **alkyne** (terminal acetylene). It can be used for the synthesis of a variety of conjugates of "partner" biomolecules, once each contains or has been grafted respectively with an azide or an alkyne group. Virtually any biomolecules can be involved, and labeling with small molecules, such as fluorescent dyes, biotin, and other groups can be readily achieved. Both azido and alkyne groups are nearly never encountered in natural biomolecules. Hence, the reaction is highly bioorthogonal and specific.

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Directions for use

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^(D) : 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](#).

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^(D) : 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 or 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, close the cap.
9. Vortex the mixture thoroughly. If significant precipitation of azide is observed, heat the vial for 3 min at 80°C, 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 °C 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 DMSO: ready to use product #FY2780

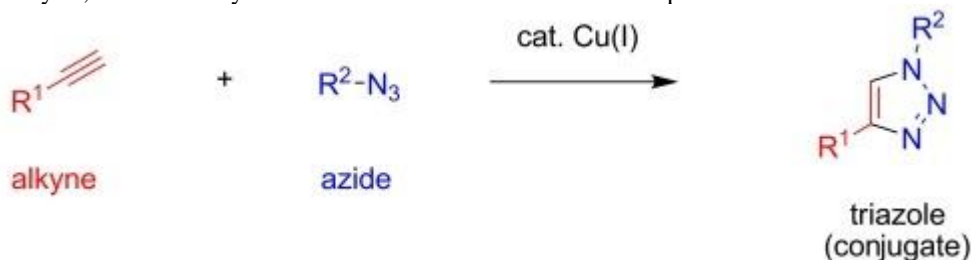
* 2M Triethylammonium Acetate Buffer, pH 7.0

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.

Technical and Scientific Information

Click Chemistry is a reaction between an alkyne and an azide yielding covalent product - 1,5-disubstituted 1,2,3-triazole. This process was popularized as the CuAAC - Cu catalyzed alkyne azide cycloaddition. It is based on copper catalysis, and the catalyst is often introduced as Cu-TBTA complex.



See also [Copper-free Click Chemistry](#) (DBCO based – SPAAC reaction).

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 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!**

Click Chemistry thus became a tool for universal modification of DNA, proteins, conjugate preparation, and fluorescent labeling. Uptima 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.

See more Chemistry reactions [XLclick](#) including protein and DNA labeling protocols.

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Related / associated products and documents

Click chemistry catalyzers and activators See Click Chemistry catalyzers (

Copper(II)-TBTA complex, 10 mM soln . #FY2780, 2x1ml

The most popular catalyst of azide – alkyne Click reaction. [Tech Sheet FY2780](#)

THPTA #APIFZA, 100mg APIFZB, 500mg APIFUC, 1g

CAS: 760952-88-3MW: 434.50 g/mol; Syn.: Tris(3-hydroxypropyltriazolylmethyl)amine.

THPTA provides benefits to Click reaction provides benefits to Click reaction similar to BTAA, plus water-solubility, that simplifies click chemistry by allowing the entire reaction to be run in water. [Tech Sheet APIZFB](#)

TTBAA #APIFU2, 100mg APIFU3, 500mg APIFU4, 1g

CAS: 1334179-85-9MW: 430.51 g/mol; Syn.: 2-(4-((bis((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetic acid.

[Tech Sheet FY2780](#)

Ascorbic acid #FZ8450, 10mg .

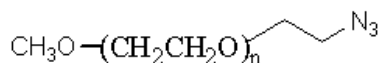
The initial catalyst proposed for azide – alkyne Click reaction. [Tech Sheet FY2780](#)

* FLUORESCENT reagents for Click Chemistry:

- Superior [FluoProbes dyes](#), activated by -Azide (i.e. FP488-Azide [#YE4970 \(protocol\)](#))
- Conventional [CyDyes dyes](#), activated by -Azide (i.e. Cy3 azide FP-EV0900 and Cy5- Azide FP-EV0910), by -Alkyne, DBCO.
- Classic dyes such as **FAM, R110, JOE TAMRA**, and **ROX**, activated by Azide, by -Alkyne, DBCO.

* AZIDE reagents – for Pegylation

mPEG_x-N₃	WU0000	1 000
Methyl-Azide	WT9980	5 000
PG1-AZ	WU0010	10 000
	WU0030	30 000



See [FT-DZ3531](#)

* ALKYNE reagents – for Pegylation

mPEG_x-Alkyne		MW
Methyl-PEG-Alkyne	FO2480	5 000
PG1-AK	FO2470	10 000
	IO5250	20 000



See [FT-DZ3531](#)

* ALKYNE reagents – for Oligonucleotides synthesis

Alkyne Amidite, 5'-terminal ZL5500

See technical sheet [ZL5500](#), [ZL5510](#)

Alkyne Amidite, hydroxyprolinol ZL5510

See technical sheet [ZL5500](#), [ZL5510](#)

* Other labels or modification reagents for Click Chemistry:

- Biotin – Azide conjugates, such as Biotin-PEG azide [FJ6751](#) and Desthiobiotin-PEG azide [FZ8440](#)

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See [BioSciences Innovations catalogue](#) and [e-search tool](#).

Ordering information

Catalog size quantities and prices may be found at <http://www.interchim.com>.

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

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