L11E

Copper-free Click Chemistry (DBCO reagents)

Conventional "Click Chemistry" requires the presence of a Cu(I) catalyst that is toxic to most organisms and thus, prevents its use in many biological systems.

The novel **Copper-free Click Chemistry** is based on the reaction of a cyclooctyne (DBCO) moiety with an azide-labeled reaction partner, known as **s**train-**p**romoted **a**lkyne **a**zide **c**ycloaddition (SPAAC). This new "Click Chemistry" is very fast at room temperature and **does not require a cytotoxic Cu(I) catalyst**. Cyclooctynes are thermostable with very narrow and **specific reactivity** toward azides, resulting in **almost quantitative yields of stable triazoles**.

This method requires to activate the biomolecule #1 with DBCO reagent, and the biomolecule #2 with azide, then to mixing the two activated biomolecules to form a conjugate.

* Features and benefits *

- **Biocompatibility** – no cytotoxic Copper catalyst required – Nice of in-vivo applications.
- **Mild conditions** – conjugation in aqueous buffered media and at low temperature
- **Stability** – DBCO and azide moieties are long term stable
- **Efficiency** – formation of a stable triazole in quantitative yield
- **Specificity** and **Bioorthogonality** –
  azide reacts only with DBCO in the presence of -NH_2, -SH, -COOH and other protein functionalities

See background information

* Interchim Bioscience's new product line of Copper-free Click Chemistry include:

- **DBCO reagents**:
  DBCO-containing Chemical Modifications Reagents :
  for introduction of functional groups, such as amines, carboxylic acids or NHS esters
  DBCO-containing Biotinylation Reagents :
  with various spacers for the introduction of Biotin moieties
  DBCO-containing Spacers : Spacer and linker building blocks containing a DBCO moiety
  DBCO-containing Fluorescent Dyes : Various fluorescent dyes modified with a DBCO group for attachment to azides
  DBCO-containing Nucleotides: Nucleotides containing a DBCO moiety for attachment to azides via Copper free click reactions

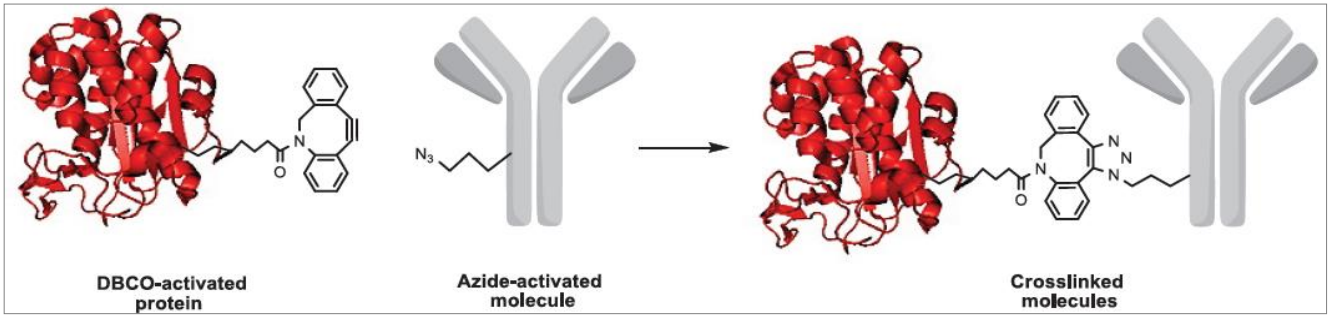
Seek also for complementary products:

- Non-Fluorescent Azides : Ferrocene, DNP, DABSYL
- Biotin Azides : incl.PEO3, SS and PEO11 spacer
- PEG and Spacer Azides
- Azides of Amino Acids
- Azides of Fluorescent Dyes: FluoProbes, Cy, Chromeo, Fluor,…
- Reagents for conventional Copper(I)-catalyzed Click Reactions, (azide, alkynes, buffers)
  such as Ethynyl-U, Ethynyl-dU, Ethynyl-dUTP and more...

■ **DBCO** Copper-Free **Click Reagents**

Our conjugation chemistry is based on the reaction of a dibenzylcyclooctyne (DBCO) linker with an azide linker to form a stable triazole. This „click reaction“ is very fast at room temperature, does not require a cytotoxic Cu(I) catalyst and creates stable triazoles. This unique covalent bond is created when DBCO, incorporated into one type of biomolecule, reacts with an azide linker, incorporated into a second biomolecule.

Unlike many conjugation reagents DBCO and azide are long term stable when attached to biomolecules. DBCO - azide conjugation chemistry is complementary and thus they react only with each other.



This method requires a three-step reaction: more information

• Step 1: Activation of biomolecule #1 with DBCO

• Step 2: Activation of biomolecule #2 with azide

• Step 3: Mixing the two activated biomolecules to form a conjugate

• Step 4 (optional): Removing excess of azide or DBCO activated biomolecule with DBCO or azide scavenger

Products Features and Benefits:

• **Stable** – forms a triazole

• **Biocompatible** – no catalyst required (e.g. Cu(I))

• **Specific** – azide reacts only with DBCO, even in presence of -NH2, -SH, -COOH or other protein functionalities
The reactive moieties do not interact with functionalities on biomolecules (bio-orthogonality)

• All reactions are carried out in aqueous buffered media, yielding **high conjugation efficienc**y.

This three step process is better than previous methods as it does not form homo-polymers and allows for more controllable formation of the desired conjugate. The DBCO and the azide linkers are available in various lengths and may be chosen to react with either an amine, thiol or carboxyl group on biomolecules. To get started, simply two reagents are required (DBCO and azide).

These crosslinkers are the most efficient and quantitative linkers available and produce high quality, easily reproducible conjugates for better performance in your assays.

DBCO-containing Reagents for Copper-free Click Reactions - DBCO-containing Chemical Modification Reagents

DBCO-Amine Dibenzylcyclooctyne-Amine	**DPQ590, 10mg / 25mg /100mg**
DBCO-Acid Dibenzylcyclooctyne-Acid	**DPQ580, 10mg / 25mg /100mg**
DBCO-NHS ester Dibenzylcyclooctyne-NHS ester	**DPQ560, 10mg / 25mg /100mg**
DBCO-S-S-NHS ester Dibenzylcyclooctyne-S-S-NHS ester	**DPQ570, 25 mg / 100mg / 1g**
DBCO-Maleimide Dibenzylcyclooctyne-Maleimide	**DQP600, 10mg / 25mg /100mg**
Sulfo-DBCO-NHS ester Sulfo-Dibenzylcyclooctyne-NHS ester	**DPQ730, 10mg / 25mg /100mg**
Sulfo-DBCO-NHS ester, Na salt	**IOJ82, 10mg /100mg**

DBCO-containing hydrophilic spacers (PEO=PEG=TEG)

DBCO-PEO$_4$-Alcohol Dibenzylcyclooctyne-PEG4-Alcohol	**DQP520, 10mg /25mg / 500mg**
DBCO-PEO$_4$-Acid Dibenzylcyclooctyne-PEG4-Acid	**DQP490, 10mg /25mg / 500mg**
DBCO-PEO$_4$-amine Dibenzylcyclooctyne-PEG4-amine	**DQP510, 10mg /25mg / 500mg**
DBCO-PEO$_5$-NHS ester Dibenzylcyclooctyne-PEG5-NHS ester	**DQP500, 10mg /25mg / 500mg**
DBCO-PEO$_4$-Maleimide	**DQP600, 10mg /25mg / 500mg**

Dibenzylcyclooctyne-PEG4-Maleimide

DBCO-containing Biotinylation Reagents

DBCO-Biotin Conjugate Dibenzylcyclooctyne-Biotin Conjugate	**DPQ840, 10mg / 25mg /100mg**
DBCO-PEG$_4$-Biotin Conjugate Dibenzylcyclooctyne-PEG4-Biotin Conjugate	**DQP720, 10mg / 25mg /100mg**
DBCO-PEG$_{12}$-Biotin Conjugate Dibenzylcyclooctyne-PEG12-Biotin Conjugate	**DQP680, 10mg / 25mg /100mg**
DBCO-S-S-PEG$_3$-Biotin Conjugate Dibenzylcyclooctyne-S-S-PEG3-Biotin Conjugate	**DQP700, 10mg / 25mg /100mg**
DBCO-S-S-PEG$_{11}$-Biotin Conjugate Dibenzylcyclooctyne-S-S-PEG11-Biotin Conjugate	**DQP690, 10mg / 25mg /100mg**
Sulfo-DBCO-Biotin Conjugate Sulfo-Dibenzylcyclooctyne-Biotin Conjugate	**DQP710, 10mg / 25mg /100mg**

DBCO-containing Fluorescent Dyes

see the technical sheet FT-DQP790

DBCO-PEO$_4$-CR110 Dibenzylcyclooctyne-Fluor 488	**FP-1C8680, 1mg / 5mg / 25mg** Abs/Em = 501/525 nm
DBCO-PEO$_4$-CR6G Dibenzylcyclooctyne-Fluor 525	Abs/Em = 522/544 nm
DBCO-PEO$_4$-TAMRA Dibenzylcyclooctyne-Fluor 545	**FP-1C8690, 1mg / 5mg / 25mg** Abs/Em = 546/565 nm
DBCO-PEO$_4$-SRB Dibenzylcyclooctyne-Fluor 568	**FP-1C8700, 1mg / 5mg / 25mg** Abs/Em = 568/584 nm
DBCO-PEO$_4$-SR101 Dibenzylcyclooctyne-Fluor 585	**FP-1C8710, 1mg / 5mg / 25mg** Abs/Em = 584/603 nm

Cy3-DBCO	**FP-1C8720, 1mg / 5mg / 25mg / 100mg**
Cy5-DBCO	**FP-1C8730, 1mg / 5mg / 25mg / 100mg**
Cy5.5-DBCO	**FP-MRV030, 2mg / 5mg / 25mg**
Cy7-DBCO	**FP-MRV040, 2mg / 5mg / 25mg**
Cy7.5-DBCO	**Inquire**

DBCO - PEGx - CYanine3, MW 3400	**1Q7070, 5mg**
Other MW on inquire: AWJT10 (2000Da), 1Q7080 (5000Da)	
DBCO - PEGx - CYanine5, MW 5000	**AWJSN0**
Other MW on inquire: AWJT20 **(**2000Da),	
DBCO - PEGx - CYanine5.5, MW 2000	**AWJT30**
Other MW on inquire	
DBCO - PEGx - CYanine7	**Inquire**

DBCO-containing Nucleotides

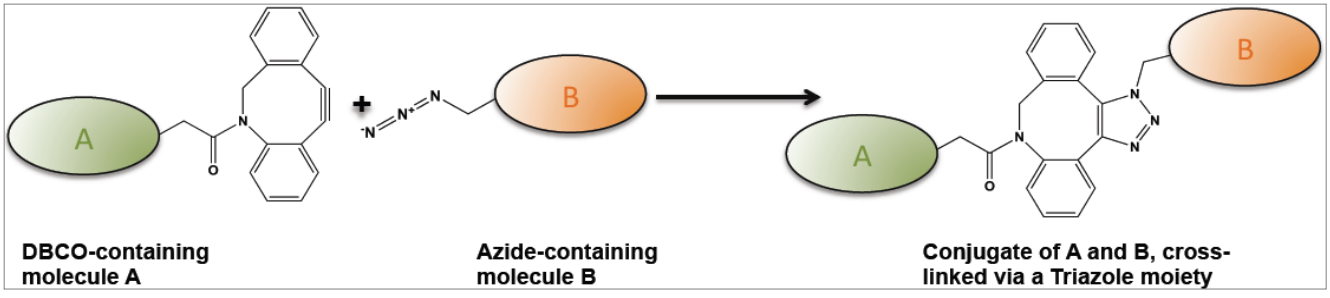
5-DBCO-dUTP 5-Dibenzylcyclooctyne-dUTP	**JO2460, 0,5 µmol / 2,5 µmol**

Please ask for other nucleotides

Copper-Free Click Reactions - Background Information

Principle

The DBCO strain-promoted or Cu(I)-free [2+3] cycloaddition strategy relies on the use of strained dibenzylcyclooctynes. Their use decreases the activation energy for the cycloaddition click reaction, enabling it to be carried out without the need for catalysis at low temperatures with an efficiency greater than that of the Cu(I)-catalyzed ligation.



Cu(I)-free ligation reaction scheme:
Diarylcyclooctyne-activated biomolecule **A** reacts with azide-activated biomolecule **B** without Cu(I) in aqueous conditions to form a stable triazole

Bertozzi and coworkers used this reaction for in vivo applications[1],[2],[3],[4].

Diarylcyclooctynes are **thermally stable** compounds with **very narrow and specific reactivity toward azides**. The ligation reaction is **very fast** and results in **almost quantitative yield** of stable triazoles.

The strain-promoted Click reaction competes with the so called Staudinger ligation (phosphine-azide). Both reactions are chemoselective and do not require copper, so both do not damage biomolecules. However, the rate of Staudinger ligation is

about 100fold lower than the rate of the DBCO cycloaddition, which makes the Staudinger ligation hardly useful for studying dynamic biological systems. Only in cases where the speed of ligation is irrelevant, both reactions can be used with about equal efficiency.

Selected References:

[1] Bertozzi *et al.* (2006) A comparative study of bioorthogonal reactions with azides. *Chem. Biol.* **1**:644.
[2] Bertozzi *et al.* (2007) Copper-free click chemistry for dynamic in vivo imaging. *Proc. Natl. Acad. Sci. U.S.A.* **104**:16793.
[3] Bertozzi *et al.* (2009) Biorthogonal Chemistry: Fishing for selectivity in a sea of functionality. *Angew. Chem. Int. Ed.* **48**:6974.
[4] Bertozzi *et al.* (2010) Rapid Cu-free click chemistry with readily synthesized biarylazacyclooctynones. *J. Am. Chem. Soc.* **132**:3688.

Related products lines

Interbiotec - BioSciences innovation – proposes a complete range of products for click biochemistry.

SPAAC'[]

Other reagents needed for Click Chemistry – AZIDES and ALKYNES

- **Azides with Fluorescent Dyes**
  FluoProbes – Azides are superior fluorescent dyes (see **caracteristics**, **protocol**)+
  CR110, CR6G, TAMRA, SRB, SR101 classic fluorescent dyes:+ +: see FT-*DQP790*
  Conventional **CyDyes**, activated by Azide , i.e. Cy3 azide FP-EV0900 and Cy5- Azide FP-*EV0910*+
  Classic dyes such as FAM, R110, JOE, TAMRA, and ROX, i.e; fluorescein-PEG-Azide *FJ0011*, Dansyl-PEG-Azide *FJ6751*+
  Others (Eterneon, Chromeo, Fluor,…): Inquire *DQP13…*+

- **Alkynes with Fluorescent Dyes**:
  CR110, CR6G, TAMRA, SRB, SR101, Cy3/5 +: see FT-*DQP790*
  Others (FluoProbes, Etternon, Chromeo, Fluor,…): Inquire +*FA030-ZC678-DQP80*

- **Biotin Azides**: see PH-BB014c
  Azide-Biotins with PEO_3, SS and PEO_{11} spacers, Desthiobiotin,… +*ZC6710* +*BT1075* +Biotin-Azide #FJ6741

- **Biotin Alkynes**: see PH-BB014c
  Acetylene-PEO_4 -Biotin :inquire *DQP65* +

- **Azides with PEO spacer(PEGs)**: see PH-BB014c
  PEO_2 to PEO_8-Azide: inquire +*DQP22, ZC684 to ZC689*

- **Alkynes with PEO spacer(PEGs)**: see PH-BB014c
  Acetylene-PEO_4 -Amine, - Acid, -NHS, -Maleimide: inquire *DQP61/3/4*+

- **Other Azides and Alkynes**: see PH-BB014c
  Ferrocene, -DNP, -DABSYL, -Pyrene *DQO50*:
  AminoOxy, Folate, Tocopherol,…: inquire +*FZ8440*

- **Azides with NucleicAcids**: see PH-BB014c +

- **Alkynes with NucleicAcids**: dUTP, dCTP, EDU, 5-EU, Phosphoramidites (CEP)…: see PH-BB014c
  +*BA0174* ; + *DQI62,DQI57,MM982,ZC68/9|DQP20/1|ZC667/8|IX028|DQO71/2/3/5* | *DQP21*

- **Alkynes and Azides of AminoAcids**: please inquire +

Other reagents needed for Click Chemistry – BUFFERS and ACTIVATORS

- **Misceallous reagents for Click Chemistry**: see PH-BB014c

Catalyzers; buffers +: see FT-*FY2780*
CuBr, TBTA, Click Solvent, AzidoAnaline,…: inquire +*ZC690,…*

Products HighLights Overview, including:
SAM reagents (Self-Assembled Monolayers)
FluoProbes labeling agents
Desalting tools – CelluSep tubings, SpectraPor tubings, GebaFlex, FloatALyser, SlideALyser,...

Information inquire

Reply by Fax : +33 (0) 4 70 03 82 60 or email at interbiotech@interchim.com

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