FT-DQP580

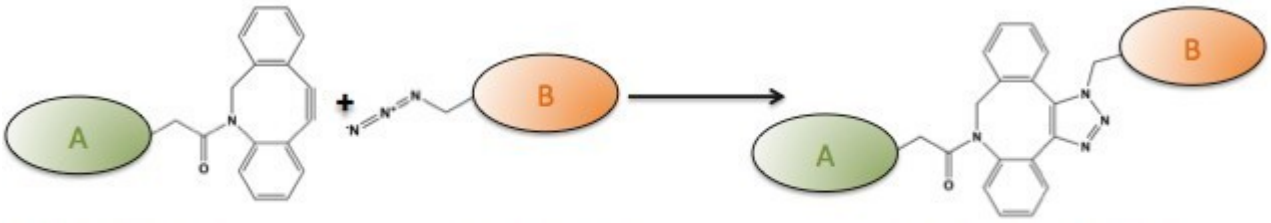
DBCO reagents for « Click Chemistry »

DBCO-containing Reagents for Copper-free Click Reactions

Introduction | A 3-step reaction | Principle of DBCO reaction | Features and benefits | Directions for use
DBCO-containing Reagents: Chemical Modification Reagents | Biotinylation Reagents | PEG Spacers | Fluorescent Dyes | Nucleotides | Linker

Introduction: Crosslinking Biomolecules using Click Reactions

The novel **Copper-free Click Chemistry** is based on the reaction of a diarylcyclooctyne moiety (DBCO, or ADIBO) with an azide-labeled reaction partner, known as **s**train-**p**romoted **a**lkyne **a**zide **c**ycloaddition (SPAAC). This new "Click reaction", unlike conventional Click Chemistry, is very fast at room temperature and **does not require a cytotoxic Cu(I) catalyst (**that is toxic to most organisms) and thus, prevents its use in many biological systems. Diarylcyclooctynes are thermostable with very narrow and specific reactivity toward azides, resulting in **almost quantitative yields of stable triazoles**.



A three-step reaction:

- 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

Principle of DBCO Reaction

The strain-promoted or Cu(I)-free cycloaddition (SPAAC) strategy relies on the use of strained cyclooctynes. Diarylcyclooctynes are thermally stable compounds with very narrow and specific reactivity toward azides. 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.

Our SPAAC conjugation chemistry is based on the reaction of a dibenzylcyclooctyne (DBCO, also known as ADIBO (= Azadibenzocyclooctyne) or DIBAC (= Dibenzoazacyclooctyne)). This „click reaction“ is very fast at room temperature, does not require a cytotoxic Cu(I) catalyst and yields almost quantitative and 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.

Products Features and Benefits:

• **Specific** – DBCO reacts only with azide, even in presence of -NH2, -SH, -COOH or other protein functionalities
DBCO group availeble functionalized by several secondary group reactive to various targets
e.g. to amine via NHS Ester- or Carboxyl-based chemistry
or to Sulfhydryl via Maleimide- and Vinyl- based chemistry
and others conjugation or modification strategies (e.g. amine-DBCA, Hydroxyl-DBCO)
or labeling strategies (Biotin, Fluorophores)

• **Bio-orthogonal** – The reactive moieties do not interact with functionalities on biomolecules

• All reactions are carried out **in aqueous buffer**ed media

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

• **Stable ligation** – forms a triazole, yielding **high conjugation efficiency**.

This three step process is better than previous methods as it d**oes 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.

Applications

- Protein-peptide conjugation
- $_{18}F$ radiolabelling
- Surface modification
- Peptide-small molecule conjugation
- Protein-oligonucleotide conjugation

DBCO-containing Copper-Free Click Reagents

for the incorporation of a DBCO moiety and subsequent conjugation to azide-containing biomolecules via Copper-free click reaction.

DBCO-containing Chemical Modification Reagents

DBCO-Acid — **1Q1000, 25mg / 100mg / 1g**
Dibenzylcyclooctyne-C4-Acid, CAS: 1353016-70-2; MW:305.11 (M)

DBCO-C_6-Acid — **DQP580, 25mg / 100mg / 1g** (ex IOJ780)
Dibenzylcyclooctyne-C6-Acid, CAS:1425485-72-8; MW: 333.38, (M)

DBCO-Amine — **DQP591, 25mg / 100mg / 1g**
Dibenzylcyclooctyne-amine; CAS:1255942-06-3; MW: 276.33 (M); Soluble in DMSO, DMF, DCM, Chloroform, THF

Sulfo-DBCO-Amine — **AXBL40, 10mg / 25mg / 100mg / 1g**
Dibenzylcyclooctyne-Sulfo-amine; ,MW: 427.47 (M); Soluble in Water, DMSO, DMF

DBCO-NHS ester — **MRV020, 25mg / 100mg / 500mg** (ex IOT800)
Dibenzylcyclooctyne-NHS ester; 30Ang.spacer; CAS:1353016-71-3; MW:402.40, (M); Soluble in DCM, DMF, DMSO, THF, Chloroform

DBCO-lc-NHS ester — **DQP560, 25mg / 100mg / 500mg**
Dibenzylcyclooctyne-C6-NHS ester; CAS:1384870-47-6; 30Ang.spacer; MW:430.46 (M); Soluble in DCM, DMF, DMSO, THF, Chloroform

DBCO-Sulfo-NHS ester — **IOJ820, 10mg / 25mg / 100mg** (ex DQP730)
Sulfo-Dibenzylcyclooctyne-NHS ester Sodium salt; CAS: 1400191-52-7; MW:532.50(509.51 free anion) (M), Soluble in DMF, DMSO, Water

DBCO-S-S-NHS ester — DQP570 — inquire
Dibenzylcyclooctyne-S-S-NHS ester

DBCO-Maleimide — **DQP600, 25mg / 100mg / 500mg**
Dibenzylcyclooctyne-Maleimide; CAS:1395786-30-7; MW: 427.45 (M); Soluble in DCM, DMF, DMSO, THF

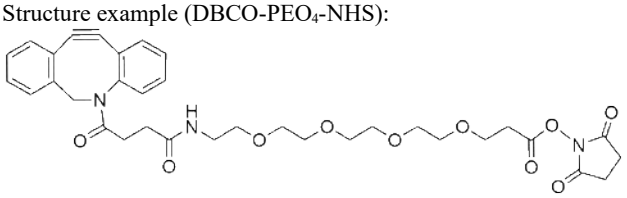
DBCO-Sulfo-Maleimide — **AXBL50, 10mg / 25mg / 100mg / 500mg**
Sulfo-Dibenzylcyclooctyne-Maleimide; MW: 578.59 (M); Soluble in Water, DMSO, DMF

DBCO-containing PEO (PEG) Spacers

The very hydrophilic synthtic PEG (PEO) spacer enhances solubility in water as well as in commonly used organic solvents of moderate polarity. The PEO_4 hydrophilic spacer will reduce or eliminate aggregation or precipitation problems when labeling antibodies and other biological molecules.

Due to its length, long spacer gives ready accessibility to the reactive groups it holds:
-the dibenzylcyclooctyne for the azide binding (click reaction).
-the other functional group: NHS for reaction to amine, Maleimide for reaction to sulfhydryl,….
The degree of DBCO incorporation (i.e. the number of DBCO per protein molecule) can be determined from the absorbance scan of the purified conjugate (235-400nm).

Structure example (DBCO-PEO_4-NHS):



DBCO-PEO_4-Acid — **MRU911, 25mg / 100mg / 500mg** (ex DQP490)
Dibenzylcyclooctyne-PEG_4-Acid; MW/ 552.62 [M]; Soluble in DMSO, DMF, DCM, Chloroform, THF

DBCO-PEO_5-Acid — **DQP491, 25mg / 100mg / 500mg**
Dibenzylcyclooctyne-PEG_4-Acid; MW:596.67 [M]; Soluble in DMSO, DMF, DCM, Chloroform, THF

DBCO-PEO_4-Amine — **DQP511, 25mg / 100mg / 500mg**
Dibenzylcyclooctyne-PEG_4-amine; ,MW: 674.75 [M]; Soluble in DMSO, DMF, DCM, water

Sulfo-DBCO-PEO_4-Amine — **IOJ780, 25mg / 100mg / 500mg**
Dibenzylcyclooctyne-Sulfo-PEG_4-amine; ,MW: 674.75 [M]; Soluble in Water, DMSO, DMF

DBCO-PEO_4-NHS ester — **MRU900, 4x2mg / 10mg / 25mg / 100mg / 500mg**
Dibenzylcyclooctyne-PEG_4-NHS ester; CAS:1427004-19-0; MW:649.68 [M]; Soluble in DCM, DMF, DMSO, THF

DBCO-PEO_5-NHS ester — **DQP500, 10mg /25mg / 100mg / 500mg**
Dibenzylcyclooctyne-PEG_5-NHS ester; MW:693.7 [M]; Soluble in DCM, DMF, DMSO, THF

DBCO-PEO_{13}-NHS ester — **AXBJE0, 4x2mg / 10mg / 25mg / 100mg / 500mg**
Dibenzylcyclooctyne-PEG_{13}-NHS ester; MW:1046.16 [M]; DMSO, DMF, THF, Acetonitrile, Dichloromethane

DBCO-PEO_4-PC-NHS ester — **AXBKX0, 10mg / 25mg / 100mg**
Photocleavable DBCO-NHS ; MW: 945.96 [M]; photoreleased >90% in 5-25 minutes using a near-UV, low intensity lamp (e.g. 365 nm lamp at 1-5 mW/cm ^{2}).

DBCO-PEO_4-Maleimide — **DQP530, 10mg /25mg / 100mg / 500mg**
Dibenzylcyclooctyne-PEG_4-Maleimide; MW: 674.76 [M]

DBCO-Sulfo-PEO_4-Maleimide — **AXBL70, 10mg /25mg / 100mg / 500mg**
Sulfo-Dibenzylcyclooctyne-PEG_4-Maleimide; MW: 825.89 [M] Soluble in Water, DMSO, DMF

DBCO-PEO_4-Bis-Sulfone — **BY141, 10mg /25mg / 100mg / 500mg**
Dibenzylcyclooctyne-PEG_4-Maleimide; MW: 1006.19 [M]; Soluble in DMSO, DMF, DCM, THF, Chloroform

DBCO-PEO_4-Hydroxyl — **DQP520, 25mg / 100mg / 500mg - inquire** (ex IOJ7811)
Dibenzylcyclooctyne-PEG_4-Alcohol; MW: 508.61 [M]; Soluble in DMSO, DMF, DCM, water

DBCO-PEO_4-DBCO — **MRU930, 10mg /25mg / 100mg / 500mg**
Dibenzylcyclooctyne-PEG_4-Dibenzylcyclooctyne; MW: 854.99 [M] . Soluble in DCM, DMF, DMSO, THF

DBCO-PEG-NHS, MW 2KDa — **1B9801, 100mg**
DBCO-PEG-NHS, MW 3KDa — **B4S4G0, 100mg**

DBCO-PEG5KDa — **IOT810, 100mg / 1g**
DBCO-PEG10KDa — **IOT820, 100mg / 1g**
DBCO-PEG20KDa — **IOT830, 100mg / 1g**
DBCO-PEG30KDa — **IOT840, 100mg / 1g**
DBCO-PEG40KDa — **IOT850, 100mg / 1g**

Solubility: DMSO, DMF, DCM, water
Store: -20°C (Shelf life: 12 months (undissolved))

DBCO-containing Linkers

H-P-Azido-Phe-OH — **SK0774, 10mg /25mg / 500mg**

DBCO-containing Biotinylation Reagents

DBCO-Biotin Conjugate — **DQP840, 10mg / 25mg /100mg**
Dibenzylcyclooctyne-Biotin

DBCO-PEG_4-Biotin Conjugate — **DQP720, 10mg / 25mg /100mg**
Dibenzylcyclooctyne-PEG_4-Biotin ; MW: (protonated) [M]; Lysis & Labeling kit: #

DBCO-PEG_{12}-Biotin Conjugate — **DQP680, 10mg / 25mg /100mg**
Dibenzylcyclooctyne-PEG_{12}-Biotin

DBCO-S-S-PEO_3-Biotin Conjugate — **DQP700, 10mg / 25mg /100mg** (ex MRV070)
Dibenzylcyclooctyne-S-S-PEG3-Biotin

DBCO-S-S-POG_{11}-Biotin Conjugate — **DQP690, 10mg / 25mg /100mg**
Dibenzylcyclooctyne-S-S-PEG_{11}-Biotin

DBCO-SulfoLink-Biotin Conjugate — **DQP710, 10mg / 25mg /100mg**
Sulfo-Dibenzylcyclooctyne-Biotin; MW: 653.77(protonated) [M]; Lysis & Labeling kit: **#MRV080**

Ask also

DBCO-PEG$_{(2000\ Da)}$-Biotin Conjugate	**IUF010, 20mg**
DBCO-PEG$_{(3400\ Da)}$-Biotin Conjugate	**1Q7050, 20mg**
DBCO-PEG$_{(5000\ Da)}$-Biotin Conjugate	**1Q7060, 20mg**

Biotin-PEO$_4$-Alkyne **Inquire**
Soluble in water

● Cleavable biotin-DBCO:
Cleavable – diazo spacer arm allows biotinylated protein targets to be released under mild conditions (by sodium dithionite (Na2S2O4))
Biocompatible – click reaction occurs efficiently under mild buffer conditions; requires no accessory reagents such as a copper catalyst or reducing agents (e.g. DTT)
Soluble – reagent easily dissolve in water-miscible solvents for subsequent dilution into aqueous reaction mixtures
Extended PEG3 Spacer – reduces aggregation, minimizes steric hindrance for affinity-binding to avidin or streptavidin

Diazo Biotin-DBCO **MRT900, 10mg / 25mg / 100mg**
MW: 973.15 (M); Soluble in DMSO, DMF

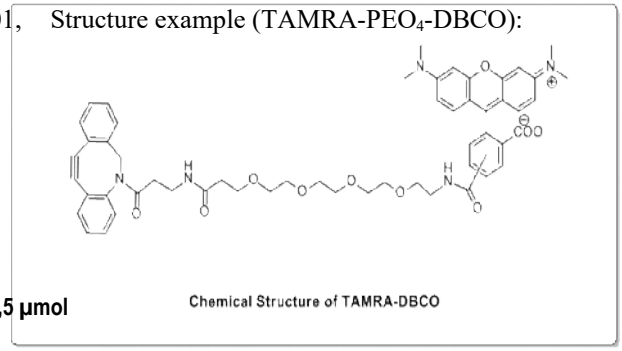
Diazo Biotin-Alkyne **MRT890, 10mg / 25mg / 100mg**
MW: 795.54 (M); Soluble in DMSO, DMF

Diazo Biotin-Azide **MRT880, 10mg / 25mg / 100mg**
MW: 711.83 (M); Soluble in DMSO, DMF

DBCO-containing Fluorescent Dyes

DBCO conjugates of fluorophores (CR110, CR6G, TAMRA, SR101, SulfoCy3/5/5.5/7) react with Azide reagents.
See FT-DQP790

Structure example (TAMRA-PEO$_4$-DBCO):



Chemical Structure of TAMRA-DBCO

DBCO-containing Nucleotides

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

DBCO-PEG$_4$-Phosphoramidite **IOL070, 10mg / 25 / 100mg**

Please ask for other nucleotides, and crosslinking agents

Selected References

Xu et al. (2011) Cytocompatible Poly(ethylene glycol)-co-polycarbonate Hydrogels Cross-Linked by Copper-Free, Strain-Promoted Click Chemistry. Chem Asian J 6:2730.

Arumugam et al. (2011) [18F]Azadibenzocyclooctyne ([18F]ADIBO): A biocompatible radioactive labeling synthon for peptides using catalyst free [3+2] cycloaddition. Bioorg Med Chem Lett 21 :6987.

Campbell-Verduyn et al. (2011) Strain-Promoted Copper-Free "Click" Chemistry for 18F Radiolabeling of Bombesin. Angew Chem Int Ed 5

Simon et al. (2012) Facile Double-Functionalization of Designed Ankyrin Repeat Proteins using Click and Thiol Chemistries. Bioconjugate Chem. 23(2):279.

Zeng et al. (2012). 64Cu Core-Labeled Nanoparticles with High Specific Activity via Metal-Free Click Chemistry. ACS Nano. 6(6):5209.

Debets et al. (2010) Aza-dibenzocyclooctynes for fast and efficient enzyme PEGylation via copper-free (3+2) cycloaddition. Chem.Commun.46:97.

Directions for use

Guidelines for use

For Handling and Storage, see label on vials. Typically, all these reagents are to be kept at -20°C for long term storage. Allow the vial to reach room temperature before opening. Avoid moisture and light. To keep unused product, especially for sensitive ones (NHS derivates), fill the vial with dry neutral gas (nitrogen or argon) before re-frezing.

Following are standard protocols. The DBCO-Azide conjugation is displayed first, but is performed typically after the activation steps by NHS or Maleimide to graft DBCO or Azide to each partner to conjugation.

●Click conjugation

●protocol to click-conjugate a DBCO-coupled molecule and a Azide coupled molecule.
See below protocols to prepare if required DBCO activated sample and/or a Azide activated sample.

Please note:
•Avoid buffers that contain azides, which can react with DBCO.
•Reactions of DBCO and azides are more efficient at high concentrations and temperatures (i.e., 4 -37°C).
Typical reaction times are less than 12 hours, however, incubating for longer can improve efficiency.

•Prepare the azide-containing sample in reaction buffer.

•Add DBCO-molecule to azide-molecule in suitable ratio.

Recommendation:
•Add 1.5 - 3 mol equivalents of DBCO-conjugate to 1 mol equivalent of azide containing protein.
Note: The ratio can by inversed if the azide activated protein is limited in quantity, while the DBCO activated partner is of highest abundance.

•Incubate the reaction mixture at room temperature for 2 - 12 hours

Note: smaller DBCO-PEG (5, 10 kDa) may require 4 - 12 hours
while larger ones (20, 30, and 40 kDa) may need 12 - 24 hours. .

•The reaction mixture is now ready for purification if required, by size exclusion chromatography or dialysis.

●DBCO stability
DBCO modified goat IgG losses about 3-5 % of its reactivity toward azides over 4 weeks at 4°C or -20°C. For long time storage azide- and thiol-containing buffers should be avoided.

●Direct Measurement of DBCO Incorporation [ref.]
The degree of DBCO incorporation (i.e. the number of DBCO per protein molecule) can be determined from the absorbance scan of the purified conjugate (235-400 nm).

Calculations for Determining Degree of DBCO Incorporation
A DBCO-IgG conjugate's degree of DBCO labeling is given by:

Number of DBCO per IgG = molarity of DBCO / Molarity of IgG

The Molarity of DBCO is calculated as follows: molarity of DBCO = (A_{309} DBCO) / E_{309} DBCO)
The molarity of IgG is calculated as follows: molarity of IgG = (A_{309} IgG) / E_{309} IgG)
where A_{309} DBCO = conjugate's absorbance at 309 nm
ε_{309} DBCO = 12 000 $M^{-1}.L.cm^{-1}$
A_{280} IgG = conjugate's corrected absorbance at 280 nm = A_{280} - (A_{309} x CF DBCO)
with CF DBCO = DBCO correction factor at 280 nm = 1.089
ε_{280} IgG ($M^{-1}.L.cm^{-1}$)= ($E_{(1\%)}$ x MW_{IgG}) / 10, whith $E_{1\%}$ = 13.6 for Goat IgG and MW =150 000

●In situ activation of a biomolecules by NHS –DBCO reagents

NHS-DBCO reagents allow to activate NH2 containing biomolecules (i.e.oprotein, peptides, amino-allyl nuleotides) for click conjugation. Succinimidy (NHS) reacts with primary and secondary amines at neutral pH.

●Procedure for **Protein Derivatization** (by using DBCO-PEG$_4$-**NHS**)

Additional Materials Required

•Water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)
•Reaction buffer: Phosphate-buffered saline (PBS) or other buffer at pH 5 - 9
•Quenching buffer: 1 M Tris-HCl, pH 8.0
•Spin Desalting Columns or Dialysis devices

•Prepare proteins in PBS.•a•

•Immediately before use, prepare 10 mM of the DBCO-PEG$_4$-NHS reagent in DMSO or DMF. •a,b,c,d•

•Add the NHS reagent to the protein sample at a final concentration of 0.5 - 2 mM. If the protein concentration is 5 mg/ml, use a 10-fold molar excess of the reagent. For samples < 5 mg/ml, use a 20- to 50-fold molar excess.

•Incubate the reaction at room temperature for 30 minutes or on ice for 2 hours.•e•

•Stop the reaction by adding Quenching Buffer to a final concentration of 50 - 100 mM Tris.

•Incubate the reaction at room temperature for 5 minutes or on ice for 15 minutes.

•Remove non-reactive reagent by dialysis or desalting

The DBCO-biomolecule is ready for Click reaction (see above).

Notes:

•a• Do not use buffers that contain primary amines, (e.g.,Tris, glycine).
Avoid buffers that contain azides, which can react with DBCO.

•b•Dissolve DBCO-PEG4-NHS ester in a dry water-miscible organic solvent such as DMSO or DMF before diluting in final reaction buffer. DBCO-PEG4-NHS ester is soluble in aqueous buffers up to 5.5 mM.

•c•NHS esters are moisture-sensitive. To avoid moisture condensation onto the product always let vial come to room temperature before opening; be careful to limit exposure to moisture and restore under an inert atmosphere. The NHS-ester moiety readily hydrolyzes and becomes non-reactive; therefore, prepare stock solutions immediately before use. Stock solutions in anhydrous solvents can be kept for several days (freeze when not in use).

•d• Hydrolysis of the NHS ester is a competing reaction. Conjugation with primary amines of proteins/peptides (i.e., acylation) is favored at near neutral pH (6 - 9) and with concentrated protein solutions. For conjugation, use non-amine-containing buffers at pH 7-9 such as PBS (20 mM sodium phosphate, 150 mM sodium chloride, pH 7.4);20 mM HEPES; 100 mM carbonate/biocarbonate; or 50 mM borate buffer.

•e• Reactions with DBCO and azides are more efficient at high concentrations and temperatures (i.e., 4 - 37°C). Typical reaction times are less than 4 hours; however, incubating for longer can improve efficiency

●In situ activation of sulfhydryl bearing biomolecules by MAL –DBCO reagents

MAL-DBCO reagent allow to activate SH containing biomolecules for click conjugation.
Maleimide (MAL) reacts with sulfhydryls at neutral pH.

Molecules, which shall react with maleimide compounds, must have free (reduced) sulfhydryls. See notes •a•

●Procedure for **Protein Derivatization** (by using DBCO-PEG4-**Maleimide**)

Additional Materials Required:

•Water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)•d•
•Reducing reagents such as Immobilized TCEP Disulfide Reducing Gel (Pierce Biotechnology)
•Reaction buffer•d•: Phosphate-buffered saline (PBS) or other sulfhydryl-free buffer at pH 6.5 - 7.5. Include 5 - 10 mM EDTA to help prevent the reoxidation of disulfides by trace divalent metals.
•(Optional): Quenching buffer: concentrated (0.5 - 1M) cycteine, DDT or other thiol containing reducing agents
•(Optional): Spin Desalting Columns or ready to use Dialysis devices (please inquire)

•Prepare sulfhydryl-containing protein, as described in note •a• (reducing; SH introduction).

•Immediately before use, weigh a small quantity of DBCO-PEG4-Maleimide and dissolve it in dimethyl-formamide (DMF) or dimethylsulfoxide (DMSO) at a 5 - 20 mM concentration. •b•

•Dissolve protein(s) in Conjugation Buffer at 0.1 mM (e.g., 5 mg in 1 ml for a 50 kDa protein).•b•

•Add DBCO-PEG$_4$-Maleimide solution to the dissolved protein(s) at 1 mM final concentration (ca ten-fold molar excess for 0.1 mM protein solution). •c•

•Incubate reaction mixture for 1 hour at room temperature or for 2 hours at 4°C.

•Quench reaction by adding Quenching Solution at 10 - 50 mM final and incubating for 15 minutes at room temperature.
Alternatively (or in addition) remove the excess non-reacted reagent by desalting or dialysis.

The DBCO-biomolecule is ready for Click reaction (see above).

Notes

•a• Reduce peptide disulfide bonds of proteins with disulfide reducing reagents such as Uptima TCEP UP242214 , i.e. high molecular weight proteins using 5 mM TCEP (1:100 dilution) for 30 minutes at room temperature, followed by TCEP removal using a desalting column (or any suitable method: gelfiltration, dialysis,…). Alternatively, use Reducing Gels provided no sulfhydryl is released.

Notes: Any remaining reducing agent (DTT, TCEP or b-mercaptoethanol) will reduce the azide, hence affect final conjugation.
Proteins (e.g., antibodies) can be inactivated by complete reduction of their disulfide bonds. Selective reduction of hinge-region disulfide bonds in IgG can be accomplished with 2-Mercaptoethylamine-HCl (2-MEA).

Alternatively, Sulfhydryls can be added to aminated molecules using N-succinimidyl S-acetylthioacetate (SATA) or 2-iminothiolane-HCl (Traut's Reagent), which modify primary amines.

•b• The maleimide group reacts predominantly with free sulfhydryls at pH 6.5 - 7.5, forming stable thioether bonds.
At pH values > 7.5, reactivity toward primary amines and hydrolysis of the maleimide groups can occur.
At pH 7, the maleimide group is 1 000 times more reactive toward a free sulfhydryl than to an amine.

•c• DBCO-PEG4-Maleimide is soluble in aqueous buffers up to 6.6 mM.
The reaction solution may appear cloudy as a result of the low aqueous solubility of DBCO-PEG4-Maleimide; usually, such solutions become clearer as the reaction proceeds. Many proteins will precipitate when the DMF or DMSO concentration exceeds 10 - 15% of the final reaction volume; if protein solubility is not an issue, there is no limit to the DMF or DMSO concentration that may be used.
•d• Do not use buffers that contain azides, which can react with DBCO.
Do not use buffers that contain sulfhydryl-containing components (e.g., DTT, TCEP or β-mercaptoethanol), because they will reduce the azide.

●In situ activation using COOH –DBCO reagents

DBCO-COOH reagents allow activating biomolecules for click conjugation. The reactivity is promoted by NHS and EDC (carbodiimide).

Applicable for:
- Protein-peptide conjugates
- Protein-oligonucleotide conjugates
- Antibody-enzyme conjugates
- Surface modification

●Procedure for Sample Activation (In situ activation to NHS esters) (by using DBCO-COOH)
Additional material required:
- Organic solvent such as methylene chloride (DCM)
- Coupling agent (e.g. EDC and NHS)

•Dissolve DBCO-Acid in dry methylene chloride (dried over 3 Å molecular sieves).

•Add 10-20% molar excess of EDC and NHS in dry methylene chloride under dry conditions.

•Stir for several hours or overnight, then evaporate the solvent and use.

Alternative: Treat the reaction mixture with a small amount of silica gel to adsorb the excess EDC and the urea by-product, then filter, evaporate and use.

The formed DBCO-NHS ester is ready for Click reaction (see above).

Troubleshooting:

Problem: No or low conjugation of DBCO and azide
•Possible reason: One or more partner molecule is not labeled

FT-DQP580

=> Confirm molecules were DBCO or Azide labeled or repeat activation process

•Possible reason: DBCO-NHS decomposed

=> Allow product to equilibrate to room temperature before opening

=> Prepare new solutions in the indicated dry solvents (and pure ones – free of amines)

=> Avoid buffers that contain primary amines such as Tris and glycine

•Possible reason: DBCO-Maleimide decomposed

=> Allow product to equilibrate to room temperature before opening

=> Prepare new solutions in the indicated dry solvents (and pure ones – free of sulfhydryls)

•Possible reason: Excess reagent not quenched or removed

=> Remove non-reacted reagent by dialysis or desalting

•Possible reason: Suboptimal reaction conditions

=> Increase incubation time

=> Optimize conjugation conditions by altering molar excess

=> Perform conjugation reactions at 37°C

Related / associated products and documents

Click Solvent (DMSO / Tert-Butanol, 3:1) #ZC6950 **Alkyne reagents** ()

Other DBCO reagents(): DBCO – fluorochromes (FT-DQP790)

See Product hightlights, Biosciences Innovation and e-search tool.

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).

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

Order on-line or Contact your local distributor

Rev.P05E-N02E