

# PEGylation via Copper-Free Click Chemistry

## Background Information

### Introduction

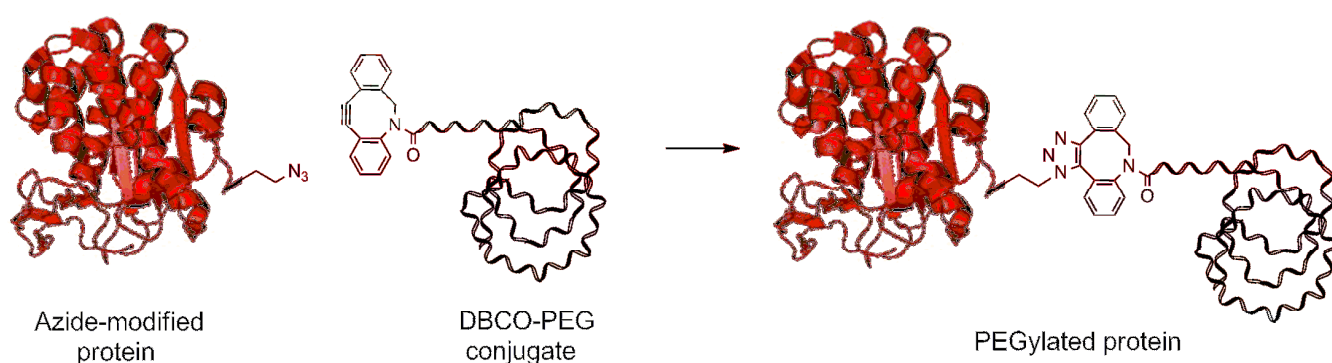
The most commonly employed method for site-specific PEGylation relies on the use of Cu-catalyzed click chemistry. The site-specific PEGylation is achieved by incorporation of an azide-containing non-natural amino acid, i.e., a homoazidoalanine into a recombinant protein that allows for site-specific conjugation with an alkyne-PEG molecule.

One major shortcoming of the Cu-catalyzed click reaction is the need for a highly toxic Cu(I) as well as Cu(II). Even in small amounts copper can damage proteins, in particular fluorescent proteins, like GFP. In addition, the presence of reducing agents, ligands and oxygen-free conditions might be required.

A method to achieve site-specific PEGylation with similar efficiency as the Cu-catalyzed click reactions while maintaining protein viability is the introduction of cyclooctynes, where the strain in the eight-membered ring allows the reaction with azides to occur in the absence of catalysts at 4°C or at room temperature. Dibenzylcyclooctynes, so-called DBCO, belong to this class of reactive cyclooctynes.

DBCO-PEG molecules allow Cu-free PEGylation of an azide-containing protein under mild reaction conditions. Concomitant, the covalent attachment of the PEG molecule to the azide residue is efficient and highly site-specific because of the inherited selectivity of click chemistry.

PEGylation of the azide-containing protein yields a single mono-PEGylated species modified at the azide residue. Because the PEGylated protein is homogeneously modified, it is readily purified and retains high biological activity.



# PEGylation via Copper-Free Click Chemistry

## Background Information

### Advantages of PEGylation via Copper-free Click Reaction

- **Efficiency** – the reaction between DBCO and azide moieties is complete in less than 24 hour at room temperature and does not require a highly toxic catalyst, e.g. Cu(I).
- **Biocompatibility** – the reaction conditions are extremely mild and do not cause protein denaturation. No metals, reducing agents or ligands are required.
- **Specificity** – azides react only with the DBCO moiety in the presence of -NH<sub>2</sub>, -SH, -COOH and other protein functionalities.
- **Biologically inertness** – the reactive moieties do not interact with functionalities on biomolecules.
- **Stability** – DBCOs and azides are stable compounds; the Copper-free click reaction is irreversible and forms a stable triazole.

### DBCO-containing PEGylation Reagents

Product	Cat. No.
DBCO-PEG 5 kDa	CLK-A118
DBCO-PEG 10 kDa	CLK-A119
DBCO-PEG 20 kDa	CLK-A120
DBCO-PEG 30 kDa	CLK-A121
DBCO-PEG 40 kDa	CLK-A122

### Recommended Assay Preparation

#### 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.

#### Copper-free click reaction:

- Prepare the azide-containing protein in reaction buffer.
- Add DBCO-PEG conjugate to azide-containing sample.  
Recommendation: Add 2 - 3 mol equivalents of DBCO-PEG conjugate to 1 mol equivalent of azide-containing protein.
- Incubate the reaction mixture at room temperature for 4 - 12 hours for smaller DBCO-PEG (5, 10 kDa) and 12 - 24 hours for larger ones (20, 30, and 40 kDa). The incubation at 4°C requires 6 - 24 hours.
- The reaction mixture is now ready for purification by size exclusion chromatography if required.

***For research use only***