DBCO LABELLING KIT

Instructions for use

PRODUCT DESCRIPTION

Product code	K DCL-3C
Intended purpose	This kit includes all the necessary reagents to perform **three DBCO-functionalizations** of amine-containing biomolecules such as proteins or peptides. It is specifically designed for use with azide-modified XanTec SPR sensor chips, including AZD, AZHC, and AZP chips, or for conjugation with the **RG-modifier** or other azide-functionalized linkers.
Kit contents	Contains buffers and chemicals to perform three DBCO conjugations • 3× DBCO-PEG_4-NHS ester, 0.13 mg/vial • 3× ZEBA™ Spin desalting columns 7 kDa MWCO, 0.5 mL • Dry DMSO, 1 mL • 10× PBS buffer; 10× dilution yields: 0.01 M $Na_xH_yPO_4$, 0.15 M NaCl, pH 7.4, 50 mL • Ethanolamine quenching buffer: 1 M ethanolamine·HCl, pH 8.5, 5 mL
Storage	• DBCO-PEG_4-NHS: Store at −20 °C desiccated over molecular sieve 4A • ZEBA™ Spin desalting columns: Store following manufacturer's instructions • 10× PBS: Store at −20 °C • DMSO: Store at −20 °C • Ethanolamine quenching buffer: Store at −20 °C
Related products	• Azide-modified sensor chips (e.g., AZD, AZHC, AZP) • RG-modifier, product code C RG-MOD-3NM

INTRODUCTION

Click chemistry has become increasingly popular among chemists because of its rapid reaction kinetics and excellent selectivity. To make this powerful bioconjugation technology accessible for SPR biosensing, XanTec offers azide (N_3)-derivatized (poly)carboxylate sensor coatings (AZD, AZHC, and AZP). These coatings enable the immobilization of dibenzocyclooctyne (DBCO)-labelled ligands in a fast and selective manner via strain-promoted azide–alkyne click chemistry (SPAAC).

Unlike EDC/NHS chemistry, DBCO/azide coupling is not time-sensitive; both reaction partners exhibit exceptional *in situ* stability for up to weeks in the pH range 4–10. The high selectivity of these reaction partners minimizes the risk of unwanted side reactions and eliminates the need for quenching steps. This makes immobilization through DBCO/azide coupling highly reliable, convenient, and flexible.

This document provides comprehensive guidance on performing DBCO functionalization of proteins and other amine-containing ligands using the XanTec DBCO labelling kit.

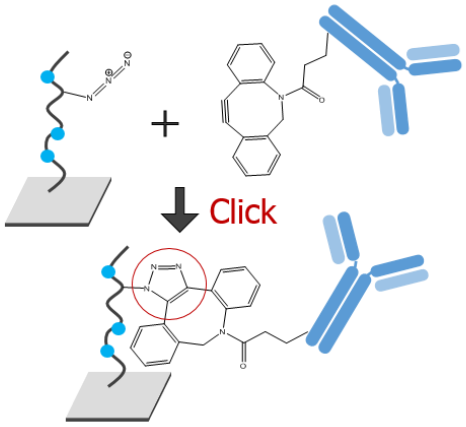

Fig. 1: Reaction between a DBCO-modified ligand and an azide-derivatized sensor coating.

Related documents

Instructions for Use – Azide-modified Sensor Chips

ADDITIONAL MATERIALS REQUIRED FOR DBCO LABELLING

A ligand bearing reactive amino groups (to be provided by the user).

PROTOCOL FOR DBCO LABELLING

Notes

The DBCO-PEG4-NHS ester is prone to hydrolysis upon exposure to moisture. After hydrolysis, it can no longer react with amine-containing ligands. Thus, store DBCO-PEG_4-NHS ester vials desiccated at -20 °C. Before opening DBCO-PEG_4-NHS ester-containing vials, equilibrate the vial to room temperature to prevent moisture condensation on the product. Dissolve the DBCO-PEG_4-NHS ester immediately before use in anhydrous (dry) DMSO. Discard any unused reagent, because it will rapidly degrade upon storage.

Avoid using buffers containing primary amines, such as Tris or glycine, because they will react with the NHS ester and deactivate the reagent. Similarly, avoid buffers containing azides, which will react with the DBCO group. If necessary, dialyze or desalt the sample to exchange it into an amine-free buffer, such as PBS. For complete removal of 0.03%–0.09% sodium azide, the desalting step using the Zeba Spin™ desalting columns should be performed at least three times.

Accurately estimating the molar excess of DBCO-PEG_4-NHS ester relative to the ligand is crucial to prevent crosslinking of DBCO-labelled proteins on the sensor chip. For proteins, which typically have multiple reactive amines (e.g., the ***N***-terminus and lysine side-chains), a degree of labelling (DOL)[1] between 0.2 and 0.5 strikes a good balance between maximizing labelled protein and minimizing multivalent labelling. At protein concentrations around 50 µM, a molar excess of DBCO-PEG_4-NHS ester of **0.4–0.8** is recommended to achieve this range. At lower protein concentrations (e.g., 15 µM), increase the molar excess to **0.8–1.0** to compensate for the increased proportion of hydrolyzed NHS ester.

Ligands containing only one reactive amine group, such as terminally amino-modified nucleic acid oligonucleotides, should be reacted with a higher molar excess of DBCO-PEG_4-NHS ester, typically in the range **10–20.**

[1] The degree of labelling (DOL) is the average number of label molecules (e.g. DBCO) attached to each target molecule.

Procedure for DBCO-labelling

1 Equilibrate all reagents of the DBCO labelling kit to room temperature.

Optional: Dilute the 10× PBS buffer with ultrapure water to produce azide-free physiological reaction buffer to conduct the DBCO-conjugation. Other physiological buffers free of amines and azides, such as HBS, work as well.

2 Ensure your ligand solution is free of sodium azide or amine-containing buffer components, such as Tris. If the ligand buffer contains these components, use the Zeba Spin™ Desalting Columns to remove them by desalting against the provided pH-neutral azide-free PBS or another suitable physiological buffer. It is recommended to perform the desalting process at least three times for optimal results. Efficient coupling requires a ligand concentration of ≥1 mg/mL.

Determine the molar extinction coefficients of the ligand at 280 nm ($\varepsilon_{P,280}$) and 309 nm ($\varepsilon_{P,309}$). This allows for precise calculation of the ligand's DOL following conjugation.

3 Calculate the required volume of DBCO-PEG_4-NHS ester solution:

$$V_{DBCO-PEG_4-NHS}\,[\mu L] = \frac{C_{ligand}\,[\mu M] \bullet V_{ligand}\,[mL] \bullet molar\ excess_{DBCO-PEG_4-NHS}}{C_{DBCO-PEG_4-NHS}\,[mM]}$$

Immediately before use, prepare a 4 mM solution of DBCO-PEG_4-NHS ester by adding 50 µL of dry DMSO to the supplied vial containing 0.13 mg of DBCO-PEG_4-NHS ester. Mix gently by pipetting up and down for 30–60 s. Add the freshly prepared DBCO-PEG_4-NHS solution to the ligand sample and mix thoroughly. Allow the reaction to proceed for 1–3 h at room temperature, or 2–8 h on ice.

4 Stop the reaction by adding 1 M ethanolamine·HCl to a final concentration of 50 mM. Incubate the mixture for 10–15 min at room temperature, or 30–60 min on ice.

5 Remove any unreacted DBCO-PEG4 reagent using a Zeba Spin™ Desalting Column.

Procedure for DBCO-labelling

6 Determine the degree of labelling of your DBCO-conjugate via UV spectroscopy. Measure the absorbance (A) of the ligand solution at 280 and 309 nm. Use the following equation to calculate the degree of labelling:

$$DOL = \frac{R \bullet \varepsilon_{P,280} - \varepsilon_{P,\,309}}{\varepsilon_{L,309} - R \bullet \varepsilon_{L,280}}$$

R = A(309 nm)/A(280 nm)
$\varepsilon_{P,280}$ = Molar extinction coefficient of the unmodified ligand at 280 nm [M^{-1} cm^{-1}]
$\varepsilon_{P,309}$ = Molar extinction coefficient of the unmodified ligand at 309 nm [M^{-1} cm^{-1}]
$\varepsilon_{L,280}$ = Molar extinction coefficient of the DBCO-linker at 280 nm = 12000 M^{-1} cm^{-1}
$\varepsilon_{L,309}$ = Molar extinction coefficient of the DBCO-linker at 309 nm = 12000 M^{-1} cm^{-1}

7 Store the DBCO-conjugate in the conditions that are optimal for the non-labelled ligand. When stored at -20°C or lower, DBCO retains its activity for several months to years.

*For **in-vitro** use only. Not for use in clinical diagnostic procedures.*

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