

DBCO Protein Labeling Kit (1mg scale)

D1510876

Storage: Room Temperature. 2-8°C. -20°C. Protect from light. For detailed storage information, please refer to the kit contents.

Shipping: Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

SKU	Prod. Name	Size
D1510875	DBCO Protein Labeling Kit (100 µg scale)	10 reactions
D1510876	DBCO Protein Labeling Kit (1mg scale)	10 reactions
D1510877	DBCO Protein Labeling Kit (5mg scale)	10 reactions

Introduction

Aladdin's DBCO Protein Labeling Kit (1mg scale) (D1510876) provides a convenient method to label proteins with dibenzylcyclooctyne (DBCO) for use in strain-promoted azide-alkyne cycloaddition (SPAAC) reactions (Figure 1). The kit uses DBCO-PEG5-NHS ester, a heterobifunctional crosslinker that readily reacts with primary amines, forming covalently attached DBCO groups that can be further reacted with an azide-labeled coupling partner to produce diverse bioconjugates. The kit includes reagents for DBCO-labeling and purification of ten 500µL samples with a concentration of 0.5–5 mg/mL. Labeling and purification can be performed in as little as 90 minutes. The kit is optimized for labeling proteins with molecular weights between 20 kDa and 150 kDa.

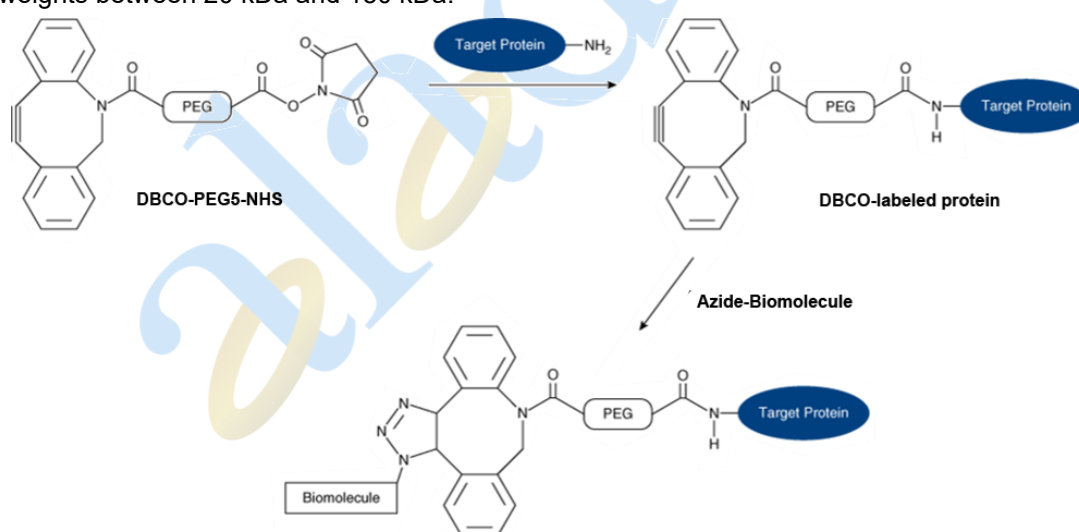


Figure 1 Schematic of SPAAC

Kit Contents

D1510876	Component	10 reactions	Storage	Quantity Per Reaction
D1510876A	DBCO-PEG5-NHS ester	7.5mg	-20°C, Store in the dark.	Prepare according to instructions
D1510876B	DMSO	1mL	RT	Prepare according to instructions
D1510876C	Purification Resin	30mL	2-8°C. Do not freeze	3mL for 1 reaction
D1510876D	Empty Spin Column	10EA	RT	1 EA for 1 reaction

Required materials not supplied

1. Variable-speed benchtop microcentrifuge.
2. 2mL microcentrifuge tubes.
3. Desired Protein for labeling (free of BSA or any carrier protein).
4. PBS buffer (pH 7.2-7.4).
5. UV-Vis spectrophotometer.

Matters needing attention

1. The purified proteins should be in a buffer that does not contain primary amines (for example, ammonium ions, Tris, glycine, ethanolamine, triethylamine, glutathione) or imidazole. All of these substances significantly inhibit protein labeling.
2. Impure proteins or proteins stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.
3. The crosslinker is moisture-sensitive. Equilibrate the vial to room temperature before opening to prevent moisture condensation inside the vial. After preparing the stock solution with the anhydrous solvent provided in the kit, it can be aliquoted and stored at -20 °C, where it remains stable for two months. Storage at -80 °C allows for even longer preservation.
4. The sample loading volume for Spin Desalting Columns must be between 400 and 700 µL to ensure optimal performance. Loading volumes outside this range may lead to lower protein recovery or inefficient removal of unconjugated reagents.
5. Do not reuse Spin Desalting Columns.

Instruction for use

1. Prepare the protein sample
 - 1.1 Prepare the target protein in a buffer (pH 7-9) free of amines, ammonium ions, or azide as these will interfere with the labeling reaction. If necessary, dialyze or exchange the target protein into an appropriate buffer, such as PBS (1×), before labeling.
 - 1.2 Prepare the protein sample at a concentration of 0.5–5 mg/mL in PBS solution. The final

volume should be $\leq 700 \mu\text{L}$.

2. Calculations

The optimal amount of DBCO-PEG5-NHS will depend on the desired degree of labeling. In general, higher protein concentrations or proteins with a greater number of accessible primary amines (e.g., lysine residues) on the surface will require less DBCO-PEG5-NHS to achieve the desired labeling density. For recommendations on DBCO:protein ratios, see Table 1.

Table 1. Recommended molar ratio of DBCO-PEG5-NHS to target protein

Protein concentration Range	Molar Ratio (DBCO:Protein)
0.5-1.0 mg/mL	40:1-20:1
1.0-5.0 mg/mL	20:1-10:1

2.1 Use the following formula to calculate the amount (in millimoles) of crosslinker to add to the sample for an X:1 molar ratio.

$$\text{mmol DBCO-NHS} = \frac{\text{mg/mL protein} \times \text{mL protein}}{\text{MW protein (mg/mmol)}} \times \text{molar ratio}$$

2.2 Use the following formula to calculate the volume (in μL) of 20 mM crosslinker to add to the sample.

$$\mu\text{L DBCO-NHS} = \text{mmol DBCO-NHS} \times \frac{1 \times 10^6 \mu\text{L}}{1\text{L}} \times \frac{1\text{L}}{20\text{mM DBCO-NHS}}$$

2.3 For 500 μL of 2 mg/mL IgG (MW = 150,000 g/mol) with 20:1 molar ratio, add 6.7 μL of 20mM DBCO-NHS to the prepared sample.

$$\text{mmol DBCO-NHS} = \frac{2\text{mg/mL protein} \times 0.5\text{mL protein}}{150000 \text{ (mg/mmol)}} \times 20 = 1.33 \times 10^{-4} \text{ mmol}$$

$$\begin{aligned} \mu\text{L DBCO-NHS} &= 1.33 \times 10^{-4} \text{ mmol DBCO-NHS} \times \frac{1 \times 10^6 \mu\text{L}}{1\text{L}} \times \frac{1\text{L}}{20\text{mM DBCO-NHS}} \\ &= 6.7 \mu\text{L} \end{aligned}$$

3. Protein labeling procedure

3.1 Equilibrate DBCO-PEG5-NHS ester to room temperature.

3.2 Just before use, reconstitute the crosslinker. Add 540 μL of DMSO to the vial of DBCO-PEG5-NHS to obtain a 20 mM stock solution. Vortex or pipette up and down until the solution is homogeneous.

3.3 Add the calculated volume of 20 mM DBCO-PEG5-NHS to the tube.

3.4 Incubate the reaction mixture for 1 hour at room temperature or 2 hours on ice.

4. Prepare the spin column

4.1 Place an empty spin column in a 15mL collection tube.

4.2 Suspend the purification resin by repeated inversion of the reagent bottle. Then add 3.0 mL of the suspension into the column and allow the resin to settle. Allow the column buffer to drain from the column by gravity. Initially, some pressure may be required to cause the first few drops of buffer to elute. Centrifuge the column at $1,000 \times g$ for 2 minutes, discard the storage buffer and return column to the same collection tube.

Note: The purification resin is supplied as a slurry in 20% ethanol, with a resin-to-ethanol volume ratio of 3:1 (v/v).

4.3 Equilibrate the column by adding 2mL of PBS to the top of the resin bed and centrifuging at $1,000 \times g$ for 2 minutes. Discard the flowthrough and repeat this step a total of 3 times.
Note: When using a fixed-angle rotor, place a mark on the side of the column that faces away from the rotor center. For all subsequent centrifugation steps, place the column in the microcentrifuge with the mark facing away from the rotor center.

5 Purify the DBCO-labeled protein

5.1 Transfer the equilibrated column into a new collection tube.

5.2 Carefully pipette the entire reaction mixture ($\leq 700 \mu\text{L}$) onto the center of the column.

Note: For samples $< 400 \mu\text{L}$, add $100 \mu\text{L}$ ultrapure water on top of the absorbed sample to increase protein recovery.

5.3 Centrifuge the column-tube assembly at $1,000 \times g$ for 2 minutes. The purified DBCO-labeled protein is in the collection tube.

6 Determine the Degree of Labeling (optional)

The efficiency of the conjugation reaction can be determined by measuring the absorbance of the protein at 280 nm and the absorbance of the DBCO group at its excitation maximum (309 nm).

6.1 For samples with high concentrations, dilute a small amount of the purified conjugate with PBS.

6.2 Measure the absorbance of the protein at 280 nm (A_{280}) and the DBCO group at 309 nm (A_{309}).

6.3 Calculate the concentration of the protein in the sample using the following formula.

$$\text{protein concentration (M)} = \frac{(A_{280} - 0.9 \times A_{309}) \times \text{Dilution factor}}{\epsilon_{\text{protein}}}$$

Where:

a) $\epsilon_{\text{protein}}$: Molar extinction coefficient of protein at 280 nm. Generally, the molar extinction coefficient of IgG at 280 nm is $203,000 \text{ cm}^{-1}\text{M}^{-1}$, and this also applies to IgA, IgD and IgE.

b) 0.90 is a correction factor for the DBCO contribution to A_{280} .

6.4 Calculate the degree of labeling (DOL) using the following formula.

$$\text{DOL} = \frac{\text{Moles of DBCO}}{\text{Moles of protein}} = \frac{A_{309} \times \text{Dilution factor}}{\epsilon_{\text{DBCO}} \times \text{protein concentration (M)}}$$

Where:

a) ϵ_{DBCO} : Molar extinction coefficient of the DBCO group at 309 nm is $12,000 \text{ M}^{-1}\text{cm}^{-1}$.

7 Conjugate the DBCO-labeled protein to an azide (optional)

7.1 Prepare the azide-labeled coupling partner (supplied separately; Cat. No. A1510878; A1510880; A1510881) in an azide-free buffer, such as PBS.

7.2 Add the prepared azide-labeled coupling partner to the DCBO-labeled protein at a 1.5 to 10 fold molar excess.

7.3 Incubate the reaction mixture for 4–12 hours at room temperature.

7.4 Depending on your application, you can use the conjugated protein immediately or purify the conjugated protein by size exclusion chromatography columns and resins.

