

## DBCO Protein Labeling Kit (5mg scale)

### D1510877

**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 (5mg scale) (D1510877) 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 2mL 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.

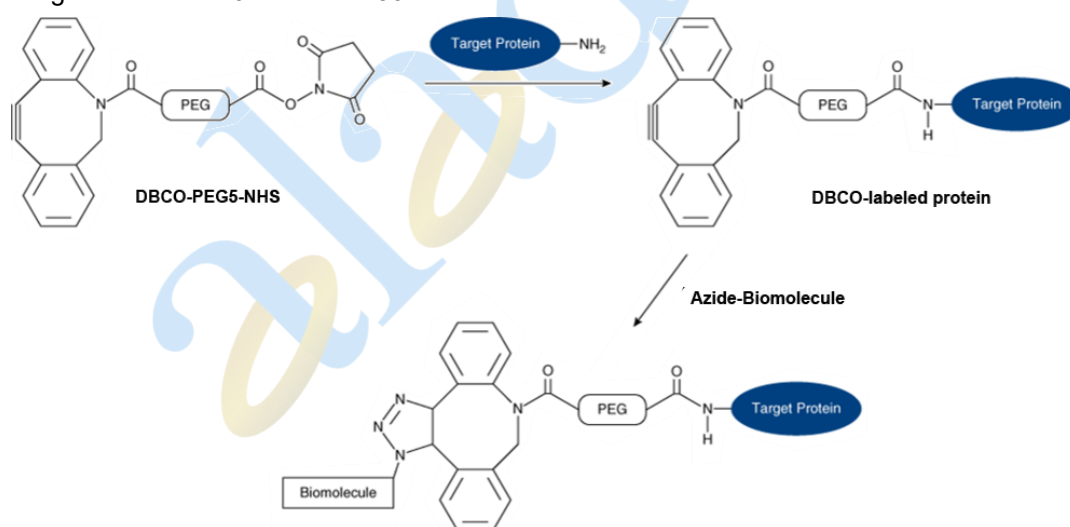


Figure 1 Schematic of SPAAC

## Kit Contents

| D1510877  | Component           | 10 reactions | Storage                   | Quantity Per Reaction             |
|-----------|---------------------|--------------|---------------------------|-----------------------------------|
| D1510877A | DBCO-PEG5-NHS ester | 15mg         | -20°C. Store in the dark. | Prepare according to instructions |
| D1510877B | DMSO                | 2mL          | RT                        | Prepare according to instructions |
| D1510877C | Purification Resin  | 140mL        | 2-8°C. Do not freeze      | 14mL for 1 reaction               |
| D1510877D | Empty Spin Column   | 10EA         | RT                        | 1 EA for 1 reaction               |

## Required materials not supplied

1. Variable-speed benchtop microcentrifuge.
2. 5mL 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 2-4mL 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 ≤4mL.

## 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  $\mu\text{L}$ ) 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 2.5mL of 2 mg/mL IgG (MW = 150,000 g/mol) with 20:1 molar ratio, add 33.5  $\mu\text{L}$  of 20 mM DBCO-NHS to the prepared sample.

$$\text{mmol DBCO-NHS} = \frac{2\text{mg/mL protein} \times 2.5\text{mL protein}}{150000 \text{ (mg/mmol)}} \times 20 = 6.7 \times 10^{-4} \text{ mmol}$$

$$\begin{aligned} \mu\text{L DBCO-NHS} &= 6.7 \times 10^{-4} \text{ mmol DBCO-NHS} \times \frac{1 \times 10^6 \mu\text{L}}{1\text{L}} \times \frac{1\text{L}}{20\text{mM DBCO-NHS}} \\ &= 33.5 \mu\text{L} \end{aligned}$$

## 3 Protein labeling procedure

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

3.2 Just before use, reconstitute the crosslinker. Add 1mL 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 50mL collection tube.

4.2 Suspend the purification resin by repeated inversion of the reagent bottle. Then add 14.0 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 10mL of PBS to the top of the resin bed and centrifuging at 1,000×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 (≤4mL) onto the center of the column.

Note: For samples < 2mL, add ultrapure water on top of the absorbed sample to increase protein recovery.

- 5.3 Centrifuge the column-tube assembly at 1,000×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)  $\epsilon_{\text{DBCO}}$ : 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.

7.5 Columns and resins.

A large, light blue, semi-transparent watermark of the Aladdin logo is oriented diagonally across the center of the page. It features the same stylized 'a's with yellow rings and a registered trademark symbol.