DBCO Labeling Kit

Item No. 702850

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Kit will arrive at two different temperatures, remove the components and store as stated below.

Item Number	Item	Quantity	Storage
401057	DBCO-NHS	5 vials/250 µg	-20°C
700621	Potassium Phosphate Buffer (500 mM, pH 7.0)	1 vial/5 ml	RT
401067	PBS (10X)	1 vial/10 ml	RT
401068	Tris-HCl (1 M, pH 8.0)	1 vial/1 ml	RT
401109	Bioconjugate Spin Columns (1 ml)	5 ea	4°C*
401164	Collection Tube	10 ea	RT

**Do not freeze!*

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's DBCO Labeling Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

E-Mail: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
2. Variable-speed benchtop microcentrifuge
3. A spectrophotometer capable of measuring absorbance at 280 and 309 nm

INTRODUCTION

Background

Copper-free click chemistry is a highly selective, bio-orthogonal reaction used to form stable covalent linkages between biomolecules under physiological conditions.[1,2] Unlike copper-catalyzed click reactions, the copper-free approach avoids the use of metal catalysts, which can generate protein-damaging reactive oxygen species (ROS), interfere with thiol-dependent enzymes, or complicate downstream applications with residual metals.[3-6] Dibenzyl cyclooctyne (DBCO) is used in a particular type of copper-free click chemistry known as strain-promoted azide-alkyne cycloaddition (SPAAC), which can be performed in cell-free, *in vitro*, or *in vivo* applications.[1] DBCO-NHS is ideal for labeling antibodies, enzymes, and other proteins, which can then be linked to azide-containing molecules, including fluorophores for imaging purposes or anticancer compounds for tumor-targeted drug delivery.[1,7-10]

About This Labeling Kit

Cayman's DBCO Labeling Kit provides a simple and convenient method to introduce a DBCO group onto proteins such as IgG or other amine-containing biomolecules. The NHS-activated DBCO reagent reacts directly with primary amines on the protein surface, enabling rapid incorporation of the DBCO moiety without affecting protein structure or function. Once labeled, the DBCO-modified protein can be used for copper-free click chemistry with any azide-functionalized partner, such as dyes, oligonucleotides, proteins, or other biomolecules. The kit provides sufficient material to label 0.05-2 mg of protein with DBCO-NHS in as little as 90 minutes.

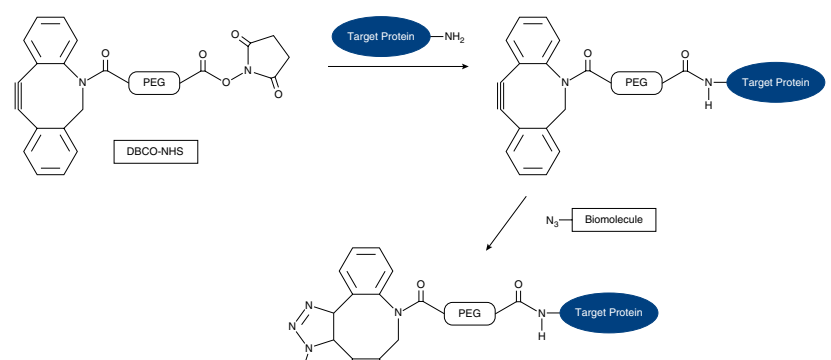

Figure 1. Schematic of SPAAC

PRE-LABELING PREPARATION

Reagent Preparation

1. **Potassium Phosphate Buffer (500 mM, pH 7.0) - (Item No. 700621)**

   This vial contains 5 ml of Potassium Phosphate Buffer (500 mM, pH 7.0). Dilute with 20 ml of ultrapure water to make 100 mM potassium phosphate buffer. Mix well before use. The diluted buffer will be stable at room temperature for six months.

2. **PBS (10X) - (Item No. 401067)**

   This vial contains 10 ml of PBS (10X). Dilute with 90 ml of ultrapure water to make PBS (1X). Mix well before use. The diluted buffer will be stable at room temperature for six months.

3. **DBCO-NHS - (Item No. 401057)**

   Each vial contains 250 µg of lyophilized DBCO-NHS. Bring the vial to room temperature. Reconstitution instructions can be found in the **Labeling Protocol** section (see page 11)

4. **Tris-HCl (1 M, pH 8.0) - (Item No. 401068)**

   Ready to use as supplied.

Sample Preparation

- 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 (1X), before labeling.
- The optimal amount of DBCO-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-NHS to achieve the desired labeling density. For recommendations on DBCO:protein ratios, see Table 1.
- Use the formula below to calculate the volume of 1.5 mM DBCO-NHS solution (µl) to add to the target protein for an **X:1** molar ratio.

$$\text{Volume DBCO-NHS needed (µl)} = \frac{\text{mg of protein}}{\text{protein MW (kDa)}} \times \text{molar ratio} \times \frac{10^6 \text{ µl/L}}{1.5 \text{ mM DBCO-NHS}}$$

Protein Concentration Range	Molar Ratio (DBCO:Protein)
0.5-1.0 mg/ml	40:1-20:1
1.0-5.0 mg/ml	20:1-15:1
5.0-10 mg/ml	15:1-10:1

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

PROTOCOL

General Information

- It is recommended that the labeling reaction be carried out at room temperature. For labeling performed at 4°C, incubation time must be determined empirically.
- For centrifugation using a fixed-angle rotor, mark one side of the spin column and position the marked side facing outward to ensure consistent resin orientation and better separation efficiency.
- The sample loading volume for Bioconjugate Spin Columns must be between 50 and 300 µl to ensure optimal performance. Loading volumes outside this range may lead to lower protein recovery or inefficient removal of unconjugated reagents. *NOTE: Do not reuse the spin columns.*

Labeling Protocol

1. Transfer the target protein into a clean microcentrifuge tube.
2. Reconstitute one vial of DBCO-NHS with 250 µl of 100 mM potassium phosphate buffer to prepare a 1.5 mM solution. Add the calculated volume of 1.5 mM DBCO-NHS to the tube. *NOTE: Reconstitute the DBCO-NHS immediately before use and discard the unused portion.*
3. Incubate for 1 hour at room temperature.
4. Quench the reaction with Tris-HCl (1 M, pH 8.0) using 10% of the reaction volume.
5. Incubate for 15 minutes at room temperature.

Purification Protocol

1. Prepare the Bioconjugate Spin Column (Item No. 401109) for purification:
    a. Twist to remove the bottom cap and place into a Collection Tube (Item No. 401164). Loosen the top cap.
    b. Centrifuge the column at 1,000 x g for 4 minutes to remove the storage buffer.
    c. Remove the top cap and add 500 µl of PBS (1X). Centrifuge at 1,000 x g for 4 minutes. Discard the flow through.
    d. Repeat step c five times, discarding the flow through after each centrifugation.
2. Place the column into a fresh collection tube and carefully add the quenched reaction (from step 4 of the labeling protocol) to the center of the resin.
3. Centrifuge the column at 1,000 x g for 6 minutes.
4. The flow through contains purified DBCO-labeled protein, which is ready for click conjugation. If not using immediately, protect from light and store at 4°C short-term or -20°C long-term.

ANALYSIS

Calculations

Determine the Degree of Labeling

The degree of labeling can be determined by measuring the absorbance values of the protein and DBCO at 280 and 309 nm, respectively. It may be necessary to dilute the DBCO-labeled protein in PBS (1X) to ensure reliable measurements. Use the equation below to calculate the degree of labelling:

$$\text{Degree of Labeling (DBCO:Protein)} = \frac{\text{Abs}_{309} \times \varepsilon_{\text{Protein}}}{\text{Abs}_{280} - (0.9 \times \text{Abs}_{309}) \times \varepsilon_{\text{DBCO}}} \times \text{Dilution Factor}$$

Where:

$\varepsilon_{\text{Protein}}$: Molar extinction coefficient of protein at 280 nm

$\varepsilon_{\text{DBCO}}$: Molar extinction coefficient of the DBCO group at 309 nm; 12,000 $M^{-1}cm^{-1}$

NOTE: The absorbance of DBCO at 280 nm is accounted for in this equation. This correction factor is 0.9 times the absorbance at 309 nm.

The degree of labeling can also be calculated by determining the molarity of DBCO-labeled protein using a Bradford assay and measuring the absorbance value of DBCO-labeled protein at 309 nM. Use the equation below to calculate the degree of labeling:

$$\text{Degree of Labeling (DBCO:Protein)} = \frac{\text{Abs}_{309}}{\text{Molarity of Protein (M)} \times \varepsilon_{\text{DBCO}}}$$

Sample Data

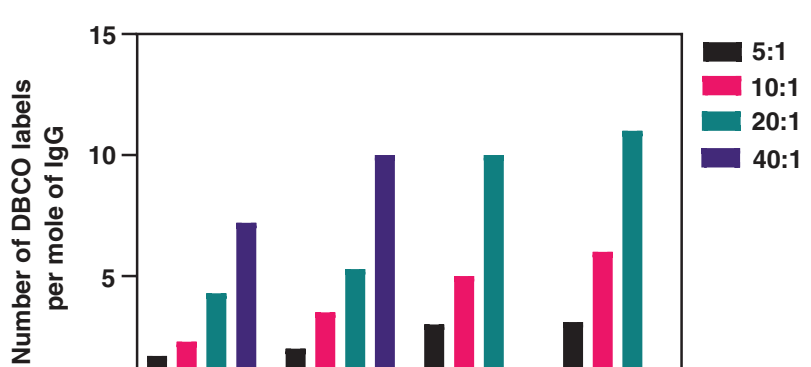

Figure 2. Labeling of rabbit IgG with DBCO-NHS. Rabbit IgG at 0.5, 1, 5, and 10 mg/ml was labeled with various molar ratios of DBCO-NHS and purified. The degree of labelling was calculated as shown in the **Calculations** section (see page 12).

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No conjugation of DBCO with protein	A. Amine or azide contamination B. Poor labeling efficiency	A. Confirm that the protein to be labeled is in an amine- and azide-free buffer B. Repeat the labeling process and increase labeling reagent, if necessary
Low or no conjugation of DBCO and azide	A. Reaction conditions were not optimized B. One or more coupling partners were not labeled	A. Optimize reaction conditions by altering molar excess of azide and increase incubation time or incubation temperature B. Confirm the biomolecules were labeled and repeat activation process

References

1. Kim, E. and Koo, H. *Chem. Sci.* **10**, 7835 (2019).
2. Mehak, Singh, G., Singh, R., *et al. RSC Adv.* **14**, 7383 (2024).
3. Li, S., Cai, H., He, J., *et al. Bioconjug. Chem.* **27**, 2315-2322 (2016).
4. Presolski, S.I., Hong, V.P., and Finn, M.G. *Current Protocols in Chemical Biology* **3**, 153-162 (2011).
5. Hatit, M.Z.C., Reichenbach, L.F., Tobin, J.M., *et al. Nat. Commun.* **9**, 4021 (2018).
6. Pickens, C.J., Johnson, S.N., Pressnall, M.M., *et al. Bioconjug. Chem.* **29**, 686-701 (2018).
7. Zhao, Z., Zhang, Z., Duan, S., *et al. Biomater. Sci.* **9(13)**, 4639-4647 (2021).
8. Rutkowska, A., Thomson, D.W., Vappiani, J., *et al. ACS Chem. Biol.* **11**, 2541-2550 (2016).
9. Wang, H., Wang, R., Cai, K., *et al. Nat. Chem. Biol.* **13(4)**, 415-424 (2017).
10. Van Deuren, V., Denis, S., Van den Eynde, R., *et al. Chem. Commun.* **60**, 14403 (2024).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.