Click Chemistry Tools
Bioconjugate Technology Company

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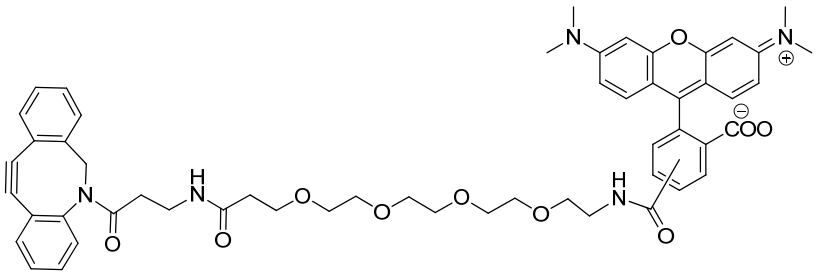
DBCO–PEG4 – TAMRA

Product No.:	A131
Product Name:	DBCO-PEG_4-TAMRA
Alternative name:	ADIBO-PEG_4-TAMRA, DBCO-PEG_4-Tetramethylrhodamine
Chemical Structure:	
Chemical Composition:	$C_{54}H_{58}N_5O_{10}$
Spectral Properties:	Abs/Em = 545/565 nm
Extinction Coefficient:	92000 $M^{-1}cm^{-1}$
Molecular Weight:	937.07
Appearance:	Red solid
Storage:	Upon receipt store at -20°C. Product shipped at ambient temperature

DBCO-PEG_4-TARMA is an azide-reactive fluorescent dye that is well suited for detection and labeling of chemically, enzymatically, or metabolically azide-modified biopolymers or peptides. The DBCO group reacts with an azide to produce a stable triazole (Figure 1), which is also referred to the Cu(I)-free or strain-promoted click reaction.

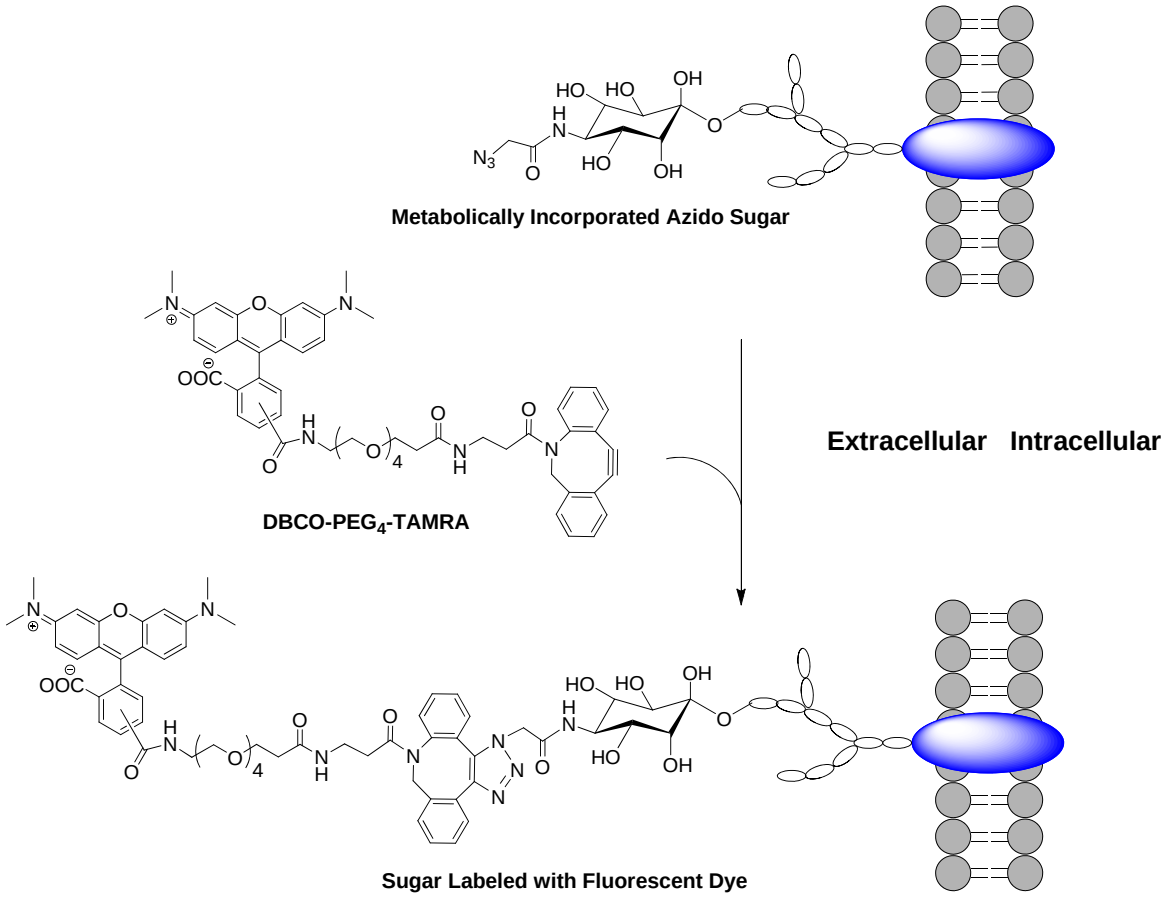


Figure 1. Reaction scheme of DBCO and azide

Live Cell Labeling

1. Grow mammalian cells in an appropriate medium with an azide-derivatized metabolite (e.g., ManNAz) at 37°C in 5% CO_2.
2. Wash the cells two times with D-PBS containing 1% FBS.
3. Prepare a 5 mM stock solution of DBCO-PEG_4-TARMA in a water-miscible solvent such as DMSO or DMF by adding 0.427 mL of solvent to 2 mg vial or 1.07 mL to a 5 mg vial, and vortex to dissolve all solid.
4. Label the azide-modified cells at room temperature in the dark for 30-60 min with 5to 30 µM of DBCO-PEG_4-TARMA in D-PBS containing 1% FBS.
5. Wash the cells four times with D-PBS containing 1% FBS.
6. Fix the cells with 4% formaldehyde in D-PBS for 20 minutes at room temperature.
7. Wash the cells with D-PBS.
8. *Optional step*: Counterstain the cells for 15 minutes at room temperature with Hoechst 33342in D-PBS.
9. Wash the cells two times with D-PBS.

10. Image the cells.

Lysing Cells

Do not use DTT, TCEP, or β-mercaptoethanol because they will reduce the azide.

1. Prepare lysis buffer (100 mM Tris buffer (pH 8.0) containing 1% (w/v) SDS).
2. *Optional step:* Add protease and phosphatase inhibitors the lysis buffer.
3. Suspend cells in the lysis buffer (50 µL lysis buffer per 10^6 cells) and heat to 75 °C. If using a 6-well plate you need 500 µL lysis buffer per100 mm dish and 200 µL lysis buffer per well.
4. Sonicate the lysate briefly to shear DNA and reduce the viscosity of the solution.
5. Vortex the lysate for 5 minutes.
6. Centrifuge the cell lysate at 16,000 *g* at 4°C for 10 minutes.
7. Transfer the supernatant to a clean tube and determine the protein concentration if required. Ideally, the protein concentration should be 1–2 mg/mL.
8. Prepare 1 M solution of iodoacetamide by adding 3 mL of DMSO to IAA labeled vial (provided with a lysis labeling kit).
9. Block cysteine thiols in lysate by addition of iodoacetamide stock solution to a final concentration of 15 mM, agitate mildly for 30 min.
10. Prepare a 5 mM stock solution of DBCO-PEG_4-TARMA by adding 0.427 mL of DBCO to 2 mg vial or 1.06 mL to 5 mg vial.
11. Label the cell lysate by addition of DBCO-PEG_4-TARMA to a final concentration of 20 µM. Protect from light and agitate mildly for 30 min at room temperature.
12. Prepare a 50 mM stock solution of stop buffer by adding 3 mL of water to Stop Reagent labeled vial (provided with a lysis labeling kit).
13. Stop reaction by addition of stop buffer to a final concentration of 100 µM, agitate briefly for 20 min.
14. Load ~10 µg of protein on 12% Tris-Tricine SDS-PAGE gel.
15. Image the gel by fluorescence scanning with detection for TAMRA (TMR), or AF 546.
16. Stain the gel with Coomassie-stain according to the manufacture's protocol, and image cells.

References

1. (a) Baskin, J. M., *et. al*. (2007). Copper-free click chemistry for dynamic *in vivo* imaging. *PNAS.*, **104(43):**16793-7. (b) Ning, X., *et al*. Visualizing metabolically labeled glycoconjugates of living cells by copper-free and fast Huisgen cycloaddition. *Anweg. Chem. Int. Ed.*, **47:**2253-5.

2. (a) Rubino F. A., *et al.* (2012). Chemoselective Modification of Viral Surfaces via Bioorthogonal Click Chemistry. *J. Vis. Exp.*, **66:**4246. (b) Yao J. Z., *et al.* (2012). Fluorophore Targeting to Cellular Proteins via Enzyme-Mediated Azide Ligation and Strain-Promoted Cycloaddition. *J. Am. Chem. Soc.*, **134:**3720−3728.

3. (a) Dieterich, D.C., *et al.* (2007). Labeling, detection and identification of newly synthesized proteomes with bioorthogonal non-canonical amino acid tagging. *Nature Protocols*, **2(3):** 532-40. (b) Best. M.D., *et al.* (2009). Click chemistry and bioorthogonal reactions: unprecedented selectivity in the labeling of biological molecules. *Biochemistry*, **48(28):**6571-84. (c) Ngo J. T., *et al.* (2012). State-selective metabolic labeling of cellular proteins. *ACS Chemical Biology*, **7(8):**1326-30.

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