USER GUIDE

Copper-Free Cell Labeling

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Materials Required

- Metabolic azide labeling reagent (e.g., Azide-sugar, https://click-chemistrytools.com/product-category/metabolic-labeling-reagents/glycosylation/)
- AZDye-DBCO (https://clickchemistrytools.com/product-category/dbco-reagents/fluorescent-probes/)
- PBS buffer (pH 7.4)
- Optional: Fixative, Hoechst 33342, DAPI, coverslips/microscope slides, mounting media.

Material Preparation

- **Azide labeling reagent:** Prepare stock solution in DMSO.
- **AZDye-DBCO Stock Solution:** Prepare 1-2 mM stock solution in DMSO or water.

Cell labeling

Growth medium, cell density, cell type variations, and other factors may influence labeling. Investigators are encouraged to determine the optimal concentration of the azide-labeling reagent as well as labeling time individually for each cell type on a small-scale first. Metabolic labeling should be carefully assessed for each cell line of interest.

1. Seed the cells at desired density.
2. Metabolically label the cells according to the research protocol.
3. Wash the cells twice with PBS.
4. Incubate with 10-20 µM AFDye-DBCO conjugate in growth media for 1 hour at 37°C.
5. Wash the cells 3 times with PBS.
6. Proceed to analysis:
   - **Flow cytometry:** Resuspend the cells in PBS containing 2% FBS and 1 mM EDTA to analyze with flow cytometry.
   - **Imaging:** Fix the cells, wash, stain with DAPI. Proceed to imaging.

   Note: If background is high – incubate the cells in AFDye-DBCO-free culture media for 1–2 hours prior the fixation.
   - **Live cell imaging:** Incubate the cells in AFDye-DBCO-free culture media for 1–2 hours. Wash one time with phenol red-free media and incubate with 10 µg/mL Hoechst 33342 in phenol-red free media for 5 minutes. Proceed to live cell imaging.

Mice labeling

1. Treat the mice with azide labeling reagent according to the research protocol (e.g., 10-50 mg/kg/day of Ac_4ManNAz for 3 days).
2. Inject 200 µL of 25 µM AFDye-DBCO (5 nmol) in tail vein, proceed to *in vivo and/or ex vivo* near-infrared or infrared fluorescence analysis.

Note: It is possible to inject cancer cells labeled with AFDye-DBCO directly in blood stream to observe tumor formation.

References

1. Kang SW, Lee S, Na JH, Yoon HI, Lee DE, Koo H, Cho YW, Kim SH, Jeong SY, Kwon IC, Choi K, Kim K. Cell Labeling and Tracking Method without Distorted Signals by Phagocytosis of Macrophages. Theranostics 2014; 4(4):420-431. doi:10.7150/thno.7265.
2. Murrey HE, Judkins JC, Am Ende CW, Ballard TE, Fang Y, Riccardi K, Di L, Guilmette ER, Schwartz JW, Fox JM, Johnson DS. Systematic Evaluation of Bioorthogonal Reactions in Live Cells with Clickable HaloTag Ligands: Implications for Intracellular Imaging. J Am Chem Soc. 2015 Sep 9;137(35):11461-75. doi: 10.1021/jacs.5b06847.
3. Yoon HI, Yhee JY, Na JH, Lee S, Lee H, Kang SW, Chang H, Ryu JH, Lee S, Kwon IC, Cho YW, Kim K. Bioorthogonal Copper Free Click Chemistry for Labeling and Tracking of Chondrocytes In Vivo. Bioconjug Chem. 2016 Apr 20;27(4):927-36. doi: 10.1021/acs.bioconjchem.6b00010.

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