USER GUIDE

Copper-Catalyzed Cell Labeling

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Materials Required

- Metabolic labeling reagent
- AZDye labeling reagent (Alkyne or Azide)
- Copper (II) Sulfate pentahydrate (https://www.sigmaaldrich.com/-catalog/product/sigald/c7631?lang=en&region=US)
- THPTA (https://clickchemistrytools.com/product/thpta/)
- Sodium ascorbate (https://www.sigmaaldrich.com/catalog/product/sigma/a4034?lang=en&region=US)
- Aminoguanidine hydrochloride (https://www.sigmaaldrich.com/catalog/product/aldrich/396494?lang=en&region=US)
- PBS buffer (pH 7.4)
- Fixative (e.g., 3.7% formaldehyde in PBS)
- Optional: Hoechst 33342, DAPI, coverslips/microscope slides, mounting media.

Material Preparation

- **Metabolic labeling reagent:** Prepare stock solution in DMSO.
- **AZDye labeling reagent (Alkyne or Azide) stock solution:** Prepare 2 mM stock solution in DMSO or water.
- **50x Copper/THPTA solution (2.5 mM CuSO4, 12.5 mM THPTA):** Weight out 31 mg of Copper (II) Sulfate Pentahydrate and 270 mg of THPTA, mix, add 50 mL of water, vortex to dissolve completely.
- **50x Aminoguanidine solution (50 mM):** Weight out 55.2 mg of Aminoguanidine hydrochloride, add 10 mL of water, vortex to dissolve completely.
- **50x Sodium Ascorbate solution (125 mM):** Weight out 20 mg of sodium ascorbate, add 2 mL of water, vortex to dissolve completely. Sodium ascorbate solution is susceptible to oxidation. We recommend always using freshly prepared solution of sodium ascorbate.
- **Click Solution (50 µM CuSO4, 250 µM THPTA, 25 µM AZDye Labeling Reagent, 1 mM Aminoguanidine, 2.5 mM sodium ascorbate in PBS):** For 1 ml of click solution, mix 20 µL of 50x Copper/THPTA, 20 µL of 50x aminoguanidine, 20 µL of 50x Sodium Ascorbate, 12.5 µL of AZDye labeling reagent (if 2 mM stock has been prepared), 927.5 µL of PBS.

Note: Prepare click solution just before use. Chill on ice for at least 10 minutes before adding to the cells.

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 metabolic 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.

Cell Analysis

1.1 Seed the cells at desired density.

1.2 Metabolically label the cells according to the research protocol.

1.3 Wash the cells twice with ice-cold PBS.

1.4 Add ice-cold **Click Solution** to the cells.

1.5 Incubate for 1-5 minutes on ice or at 4°C.

1.6 Wash the cells twice with PBS.

1.7 (Optional) Proceed to live cell imaging or flow cytometry.

1.8 Fix the cells with fixative for 10 minutes at room temperature.

1.9 Remove the fixative and wash the cells 3 times with PBS.

1.10 (Optional) Stain nuclei with Hoechst or DAPI.

1.11 Wash the cells 3 times with PBS.

1.12 Proceed to imaging or flow cytometry.

If dual labeling is desired: Wash the cells twice with growth media after Step 1.5. Return the cells to growth media containing metabolic labeling reagent for 20 hours.

References

Hong V, Steinmetz NF, Manchester M, Finn MG. Labeling live cells by copper-catalyzed alkyne--azide click chemistry. Bioconjug Chem. 2010 Oct 20;21(10):1912-6. doi: 10.1021/bc100272z.

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