USER GUIDE

Cell Lysate Labeling

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This is a general protocol for labeling cell lysate through copper-catalyzed click reaction with any azide or alkyne click detection reagent. This protocol may be used as a starting point for optimization of particular click chemistry procedures. Slight adjustment of azide or alkyne click detection reagent concentrations might be required in case of high background or low signal intensity. Final concentration of an azide or alkyne detection reagent may range from 2 µM to 40 µM. Final concentrations below or above this range are also possible, and should be optimized per the specific application. We recommend starting with a final concentration of 20 µM, and titrating this amount down in case of high background.

Materials Required:

azide or alkyne detection reagent, THPTA, copper (II) sulfate pentahydrate, sodium ascorbate, PBS buffer (pH 7.4).

Material Preparation:

- **100 mM THPTA ligand in aqueous buffer or water:** Prepare 100 mM stock solution in water, for example add 2.3 mL of water to 100 mg vial of THPTA, sonicate to dissolve.
- **Azide or alkyne detection reagent:** Prepare 1 mM solution in DMSO or water. Example: Add 750 µL of water to 1 mg of AFDye 488 Azide, swirl or sonicate to make a solution.
- **20 mM copper (II) sulfate solution.** Dissolve 0.25 g of Copper (II) Sulfate Pentahydrate in 50 mL of water, vortex to dissolve completely.
- **Reducing Agent:** Dissolve 20 mg of sodium ascorbate in 0.36 mL of deionized water. Vortex until completely dissolved. Sodium ascorbate solution is susceptible to oxidation. We recommend always using freshly prepared solution of sodium ascorbate.

Click Reaction

Note: 200 µL of the reaction cocktail is used per coverslip. A smaller volume can be used if the remaining reaction components are maintained at the same ratios.

1. For each azide or alkyne- modified protein lysate sample, add the following to a 1.5 mL microfuge tube, then vortex briefly to mix.
   - 50 µL protein lysate (1-5 mg/mL) in protein extraction buffer
   - 100 µL PBS buffer
   - 4 µL corresponding azide or alkyne detection reagent (20 µM final concentration)
2. Add 10 µL of 100 mM THPTA solution, vortex briefly to mix.
3. Add 10 µL of 20 mM $CuSO_4$ solution, vortex briefly to mix.
4. Add 10 µL of 300 mM sodium ascorbate solution to initiate click reaction, vortex briefly to mix.
5. Protect reaction from light and allow click reaction to incubate for 30 minutes at room temperature. Longer reaction time might improve labeling efficiency.
6. Proteins in lysate are now click labeled and ready for downstream processing and/or analysis.

Preparation of Samples for Gel Analysis

1. Add 600 µL methanol to 200 µL reaction mixture, vortex briefly.
2. Add 150 µL chloroform, vortex briefly.
3. Add 400 µL dH_2O, vortex briefly.
4. Centrifuge for 5 minutes at 13,000-20,000 g, carefully remove upper aqueous layer without disturbing interface layer containing proteins. Note-upper aqueous layer may either be colorless or contain color depending on detection reagent.
5. Add 450 µL methanol, vortex briefly.
6. Centrifuge for 5 minutes at 13,000-20,000 g to pellet protein. Carefully, remove and discard supernatant.
7. Add 450 µL methanol, vortex briefly. Repeat step 6.
8. Open the lid to microfuge tube and allow protein pellet to air-dry for at least 15 minutes.
9. Cap and store labeled sample at -20°C until ready for use.

Labeling of Live Cells

See Hong, V., et al (ref. 5) for detailed protocol and suggestions.

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