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Two Color TEM Labeling using Silver-Gold Intensification of DAB and CuDAB2

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Mason Mackey[1], Stephen Adams[1], Mark Ellisman[1]

[1]UCSD

NCMIR@UCSD

National Center for Microscopy and Imaging Research
University of California, San Diego

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We use this protocol and it's working

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Abstract

This method is use for two color labeling of cell organelles for transmission electro microscopy. The color TEM labeling used Silver-Gold Intensification on polymerized DAB from photooxidation of miniSOG-mitomatrix and CuDAB2 labeling on DNA from oxidation of FeTAML-azide. The labeling were confirmed by EELS using a Gatan Imaging Filter on a ThermoFisher Talos 200C G2 transmission electron microscope at 200kV.

Guidelines

The method is use for two color labeling of cell organelles for transmission electro microscopy. 80 um sections were collected and metals were verified using EELS from a Gatan Imaging Filter (GIF).

Materials

1. HEK293T cells
2. MatTek dishes containing 35mm glass bottom No. 0 coverslips coated with poly-d-lysine (P35GC-0-14C, 3. MatTek Corporation)
3. EdU Quantity: 50 mg (A10044, ThermoFisher)
4. 100 mM NaCl 50 mM bicine pH 8.3
5. 2 mM Cu-DAB2
6. 2% EM grade glutaraldehyde (18426, Ted Pella Incorporated)
7. 1X PBS
8. FeTAML-azide
9. 2% sodium acetate
10. Heating plate
11. 2.5% sodium thiosulphate
12. 5% silver nitrate
13. 0.05% tetrachlorogold (III) acid trihydrate
14. 3% hexamethylenetetramine
15. 2.5% disodium tetraborate
16. Lipofectamine 3000 (Life Technologies)
17. standard GFPex475 filter set
18. 1X PBS

Safety warnings

Use PPE to protect from toxic chemicals.

Fixation and photooxidation of miniSOG-Mitomatrix.

1 HEK293T cells are cultured on imaging plates containing poly-d-lysine coated glass bottom No. 0 coverslips (P35GC-0-14C, MatTek Corporation).

2 Cells are transiently transfected with miniSOG-mitomatrix fusion using Lipofectamine 3000 (Life Technologies). MiniSOG is fused to N-terminal of mitomatrix. Cells also are incubated with 10 µM EdU for 12hours.

3 Fix cells with 2% EM grade glutaraldehyde (18426, Ted Pella Incorporated) in 1XPBS, pH 7.2 (18851, Ted Pella Incorporated) containing for 5 minutes at 37^{o}C and then on ice for 55 minutes.

3.1 *Rinse once with fix after removing cell culture media. Afterwards add the fix for the total duration.

4 Wash 5X with 1X PBS buffer pH 7.2 for 2 minutes each on ice.

5 Blocking step for miniSOG: Block solution: (20 mM glycine, 10 mM KCN and 10 mM aminotriazole).
Cells are blocked for 20 minutes on ice.

6 Wash 2X with 1X PBS, pH 7.2 for 2 minutes each on ice.

7 For mSOG, regions of interest are identified by standard GFPex475 filter set at light intensity of 10% and for photooxidation of DAB in 1X PBS at pH 7.2 the light intensity is set at 100%. A photooxidation reaction with DAB in 1X PBS at pH 7.2 until the desired brown intensity color from the precipitate for 2-5 minutes.

8 Wash 5X with 1X PBS, pH 7.2 for 2 minutes each on ice.

Gold enhancement on polymerized DAB.

9 Wash 3X with 2% sodium acetate for 1 minutes each at room temperature.

10 Cells in MatTek plate are prewarmed at 60°C for 10 min in 2% sodium acetate.

11 Make enhancement solutions containing 3% **hexamethylenetetramine** in ddH_2O, 5% **silver nitrate** in ddH_2O, and 2.5% **disodium tetraborate** in ddH_2O, mixed in a ratio of 20:1:2, prewarm solution for 10 minutes at 60^{o}C, added to the cells and incubated for 8-11 min at 60°C.

12 Wash 5X3 min with 2% sodium acetate at room temperature.

13 Incubate cells with 0.05% **tetrachlorogold (III) acid trihydrate** in ddH_2O for 5 minutes at room temperature.

14 Wash 5X3 min with 2% sodium acetate at room temperature.

15 Incubated with 2.5% **sodium thiosulphate** in ddH_2O for 4 min at room temperature.

16 Wash 1X3 min with 2% sodium acetate room temperature.

17 Wash 5X1 min with 1XPBS on ice.

Click reaction of Fe-TAML-azide to EdU

18 The click reaction of the cells were carried out at room temperature and protected from light by using a mixture of 28 µM Fe-TAML azide solution, freshly prepared from 900 µl click buffer (100 mM NaCl 50 mM Na·HEPES pH 7.6), 10.0 µl CuSO4 (100 mM in water), 0.1% Saponin (Sigma, S-4521) and the reaction was initiated with 100 μl of freshly prepared aqueous sodium ascorbate (100 mM).

19 After 30 minutes, a second 100 μL aliquot of newly prepared aqueous sodium ascorbate (100 mM) was added for another 30 minutes incubation.

20 Cells are washed with PBS pH 7.4 (5 × 1 min) at room temperature.

21 Cells are washed with 100 mM NaCl 50 mM Na·Bicine pH 8.3 (5 × 1 min).

Copper-DAB polymerization

22 2 mM Cu-DAB2 solutions in 100 mM NaCl 50 mM bicine pH 8.3 were prepared similarly to previously reported solutions of Ln-DAB2 in cacodylate buffer (Adams et al 2016) by substituting with stock solutions of 0.1M CuSO4, 1M-bicine buffer pH 8.3 and 1M-NaCl.

23 10 µl of H_2O_2 from a 30% stock solution was added to 10ml of the 2 mM Cu-DAB2.

24 The Cu-DAB2 reaction ran for one hour on the cells.

25 Cells are washed with 100 mM NaCl 50 mM Na·Bicine pH 8.3 (5 × 1 min).

26 Process for TEM.

Protocol references

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