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Mar 16, 2022

Photo-oxidation Using MiniSOG with EM Preparation of Transfected Culture Cells V.1

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dx.doi.org/10.17504/protocols.io.eq2lynj8pvx9/v1

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Citation: Mason Mackey, Xiaokun Shu, Varda Lev-Ram, Thomas J. Deerinck, Yingchuan Qi, Ericka B. Ramko, Michael W. Davidson, Yishi Jin, Mark H. Ellisman, Roger Y. Tsien Photo-oxidation Using MiniSOG with EM Preparation of Transfected Culture Cells https://dx.doi.org/10.17504/protocols.io.eq2lynj8pvx9/v1

Abstract taken from Plos Biology Journal: *A Genetically Encoded Tag for Correlated Light and Electron Microscopy of Intact Cells, Tissues, and Organisms*

Electron microscopy (EM) achieves the highest spatial resolution in protein localization, but specific protein EM labeling has lacked generally applicable genetically encoded tags for in situ visualization in cells and tissues. Here we introduce “miniSOG” (for mini Singlet Oxygen Generator), a fluorescent flavoprotein engineered from *Arabidopsis* phototropin 2. MiniSOG contains 106 amino acids, less than half the size of Green Fluorescent Protein. Illumination of miniSOG generates sufficient singlet oxygen to locally catalyze the polymerization of diaminobenzidine into an osmiophilic reaction product resolvable by EM. MiniSOG fusions to many well-characterized proteins localize correctly in mammalian cells, intact nematodes, and rodents, enabling correlated fluorescence and EM from large volumes of tissue after strong aldehyde fixation, without the need for exogenous ligands, probes, or destructive permeabilizing detergents. MiniSOG permits high quality ultrastructural preservation and 3-dimensional protein localization via electron tomography or serial section block face scanning electron microscopy. EM shows that miniSOG-tagged SynCAM1 is presynaptic in cultured cortical neurons, whereas miniSOG-tagged SynCAM2 is postsynaptic in culture and in intact mice. Thus SynCAM1 and SynCAM2 could be heterophilic partners. MiniSOG may do for EM what Green Fluorescent Protein did for fluorescence microscopy.

https://doi.org/10.1371/journal.pbio.1001041

DOI

dx.doi.org/10.17504/protocols.io.eq2lynj8pvx9/v1

Mason Mackey, Xiaokun Shu, Varda Lev-Ram, Thomas J. Deerinck, Yingchuan Qi, Ericka B. Ramko, Michael W. Davidson, Yishi Jin, Mark H. Ellisman, Roger Y. Tsien 2022. Photo-oxidation Using MiniSOG with EM Preparation of Transfected Culture Cells. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.eq2lynj8pvx9/v1

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https://dx.doi.org/10.17504/protocols.io.eq2lynj8pvx9/v1

NIH/National Institute of Neurological Disorders and Stroke
Grant ID: U24NS120055

NIH/National Institute of General Medical Sciences
Grant ID: R24GM137200

NIH grants
Grant ID: GM086197

NIH grants
Grant ID: NS035546

NIH
Grant ID: P41-RR004050

protocol

Shu X, Lev-Ram V, Deerinck TJ, Qi Y, Ramko EB, Davidson MW, et al. (2011) A Genetically Encoded Tag for Correlated Light and Electron Microscopy of Intact Cells, Tissues, and Organisms. PLoS Biol 9(4): e1001041. https://doi.org/10.1371/journal.pbio.1001041

photo-oxidation, MiniSOG, Preparation of Transfected Cultured Cells, UCSD, NCMIR

protocol ,

Mar 02, 2022

Mar 16, 2022

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List of Buffers and Solutions Used:

- *0.1M Sodium Cacodylate Buffer Recipe for 100ml:
- 65.7 ml of DDH2O + 1ml of 0.204 M CaCl2 + 33.3ml of 0.3M sodium cacodylate pH 7.4

- DAB solution
- (0.0052grams DAB in 100µl 1.0N HCL and then add 900µL DDH20). Sonicate for 20 minutes. Add 9.0mL 0.1M sodium cacodylate buffer pH 7.4. Place on Ice. Remove solution after contrast development.

- Durcupan Epoxy Resin Components
1. 11.4 grams
2. 10 grams
3. 0.3 grams
4. 0.1 grams

Mix well to the point it homogenous and minimize air bubbles. Let sit for 20 minutes before using.

Durcupan resin epoxy in liquid form is a carcinogen, please wear proper PPE.

Sodium Cacodylate contains arsenic, therefore it is toxic. Wear proper PPE.

Uranyl acetate and glutaraldehyde is toxic, wear proper PPE.

Osmium tetroxides are very powerful oxidizers, they must be used in the fume hood.

1 Cells are grown on glass bottom MatTek culture dishes. (P35G-0-14-C, MatTek Corp)

2 Transfected Cells are fixed with 37oC 2% glutaraldehyde in *0.1M sodium cacodylate buffer pH 7.4 with 2.0 mM CaCl2 for 5 minutes at room temp and then for 55 minutes on ice.

3 Wash 5X with 0.1M sodium cacodylate buffer pH 7.4 with 2.0 mM CaCl2 for 2 minutes each on ice.

4 Block with 50 mM glycine, 10 mM KCN, 10 mM aminotriazole and 0.4mM H2O2 in 0.1M sodium cacodylate buffer pH 7.4 with 2.0 mM CaCl2 for 20 minutes on ice. Added H2O2 just before using.

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5 Wash 2X with 0.1M sodium cacodylate buffer pH 7.4 with 2.0 mM CaCl2 for 2 minutes on ice.

6 Collect confocal fluorescent images. (Excitation beam 488nm)

7 Add DAB with 0.22um Millex 33mm PES sterile filter. Let sit for 5 minutes.

8 Photooxidation using GFP filter (Ex:450-490nm, DM:510nm and Em:515nm LP) with intense light from a 150W xenon lamp with a stream of pure oxygen gently bubbling the DAB solution.

9 Wash 5X with 0.1M sodium cacodylate buffer pH 7.4 with 2.0 mM CaCl2 for 2 minutes on ice.

10 Post-fix cells with reduced 1-2% osmium tetroxides (0.8% potassium ferrocyanide + 2.0 mM CaCl2) in 0.1M sodium cacodylate buffer pH 7.4 for 30 minutes on ice.

11 Wash 3X with 0.1M sodium cacodylate buffer pH 7.4 with 2.0 mM CaCl2 for 1 minutes on ice.

12 Wash 5X with cold DDH2O, dehydrate 20, 30, 70, 90, 100% ethanol on ice one minute each.

13 Dehydrate with 100% dry ethanol 3X 1 minute each at room temperature.

14 Infiltrated with (1:1 ratio) 100% dry ethanol and Durcupan epoxy resin for 30 minutes. with lid on and place on rocker. Make sure both components are mixed completely where the mixture is homogenous.

15 Add 100% Durcupan epoxy resin overnight.

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16 Next day, 3X Durcupan epoxy resin for one hour each and then place in vacuum oven at 60Co for 48 hours.

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https://dx.doi.org/10.17504/protocols.io.eq2lynj8pvx9/v1