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Lanthanum DAB metals, Ln-DAB2 labeling of APEX2

Stephen R. Adams[1,2], Mason R. Mackey[2], Alice Ting[3,4,5,6], Mark Ellisman[2,7,8], Thomas Deerinck[2]

[1]Department of Pharmacology, University of California, San Diego, La Jolla CA;
[2]National Center for Microscopy and Imaging Research, University of California, San Diego, La Jolla CA;
[3]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA ;
[4]Department of Genetics, Stanford University, Stanford, California, USA;
[5]Department of Biology, Stanford University, Stanford, California, USA;
[6]Department of Chemistry, Stanford University, Stanford, California, USA;
[7]Department of Neurosciences, University of California at San Diego, La Jolla, California, USA;
[8]Department of Bioengineering, University of California at San Diego, La Jolla, California, USA

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NCMIR@UCSD

NCMIR
University of California, San Diego

ABSTRACT

This protocol uses APEX2, an engineered peroxidase that genetically targets a cellular region of interest, to oxidatively polymerize Ln-DAB2, (lanthanum chelates conjugated to DAB), using hydrogen peroxide. We use these precipitated lanthanum metals to identify the labeled regions of interest by using EELS and elemental mapping.

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lanthanium, DAB metals, APEX2, HEK293T cells

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SAFETY WARNINGS

Wear PPE.

BEFORE STARTING

Buffer Recipe: (65.7 ml of DDH2O + 1ml of 0.204 M CaCl2 + 33.3ml of 0.3M sodium cacodylate pH 7.4)

Fixative in conical tube: 2.0 ml from 25% EM grade glutaraldehyde stock vial, 1.0 ml of 0.3M stock sodium cacodylate buffer and 22.0 ml of 0.1M sodium cacodylate buffer, pH

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7.4.

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 either APEX2-H2B or mitochondrial matrix-APEX2 fusion using Lipofectamine 3000 (Life Technologies). APEX2 is fused to N-terminal of H2B and to C-terminal of mito matrix.

3 After 16 hours transfection, cells are fixed with 2% EM grade glutaraldehyde (18426, Ted Pella Incorporated) in 0.1M sodium cacodylate buffer, pH 7.4 (18851, Ted Pella Incorporated) containing 2 mM $CaCl_2$ for 5 minutes at 37°C and then on ice for 55 minutes.

4 Fixative is removed and cells are rinsed with 0.1M sodium cacodylate buffer, pH 7.4 (5X1min) on ice.

5 Add 20mM glycine in 0.10M sodium cacodylate buffer, pH 7.4 for 15-20 minutes.

6 Rinse cells 2x for 1 minute with the 0.10M sodium cacodylate buffer, pH 7.4.

7 On a set plates, an enzymatic reaction with Ln-DAB_2 with 4 mM H_2O_2 (from 30%) in 0.1M sodium cacodylate buffer at pH 7.4 until the desired brown intensity color from the precipitate, between 2 to 30 minutes. (1ul of 9.8M H_2O_2 added in 2500 ul or 2.5 ml Ln-DAB_2 solution). **See making of Ln-DAB_2 solutions.**

8 After reactions, all plates of cells are rinsed with 0.1M sodium cacodylate buffer, pH 7.4 containing 2 mM $CaCl_2$ (5X1min) on ice.

9 Cells are post-fixed with either 0.01% ruthenium tetroxide (20700-05, Electron Microscopy Sciences) or 1% reduced osmium tetroxide (19150, Electron Microscopy Sciences) containing 2 mM $CaCl_2$ and 0.8% potassium ferrocyanide in 0.1M sodium cacodylate buffer, pH 7.4 for 30 minutes.

After 15 minutes, the 0.01% ruthenium tetroxide solution should be removed

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9.1 and replaced with new 0.01% ruthenium tetroxide solution.

10 Post fixative is removed from cells and are rinsed with 0.1M sodium cacodylate buffer at pH 7.4 (5X1min) on ice.

11 Cells are washed with ddH_2O (5X1min) on ice.

12 An ice-cold graded dehydration ethanol series of 20%, 50%, 70%, 90%, 100% (anhydrous) for one minute each and 2X 100% (anhydrous) at room temperature for 1 minute each.

13 Cells were infiltrated with one part Durcupan ACM epoxy resin (44610, Sigma-Aldrich) to one part anhydrous ethanol for 30 minutes.

14 Plates are changed three times with 100% Durcupan resin for 1 hours each with the first change having the lid only partly covering the top of the plate, a final change of Durcupan resin and immediately placing the plate without the lid in a vacuum oven at 60°C for 48 hours to harden.

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