



Sep 16, 2022

Lanthanum DAB metals, Ln-DAB2 labeling of APEX2

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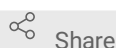
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dx.doi.org/10.17504/protocols.io.e6nvwkoe2vmk/v1

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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.

DOI

dx.doi.org/10.17504/protocols.io.e6nvwkoe2vmk/v1

PROTOCOL CITATION

Stephen R. Adams, Mason R. Mackey, Alice Ting, Mark Ellisman, Thomas Deerinck 2022. Lanthanum DAB metals, Ln-DAB2 labeling of APEX2.

protocols.io

<https://protocols.io/view/lanthanum-dab-metals-ln-dab2-labeling-of-apex2-b88erzte>



FUNDERS ACKNOWLEDGEMENT

UCSD Graduate Training Programs in Cellular and Molecular Pharmacology

Grant ID: (T32 GM007752)(SP)

Neuroplasticity of Aging

Grant ID: (T32 AG000216)(SP)

NIH

Grant ID: R24GM137200

NIH

Grant ID: P41 GM103412

NIH

Grant ID: R01GM086197

4D Stem Grant

Grant ID: R01GM138780

KEYWORDS

lanthanum, DAB metals, APEX2, HEK293T cells

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CREATED

May 11, 2022

LAST MODIFIED

Sep 16, 2022

PROTOCOL INTEGER ID

62438

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

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 (5X1 min) 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-DAB₂ 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-DAB₂ solution). **See making of Ln-DAB₂ solutions.**
- 8 After reactions, all plates of cells are rinsed with 0.1M sodium cacodylate buffer, pH 7.4 containing 2 mM CaCl_2 (5X1 min) 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

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 ddH₂O (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.