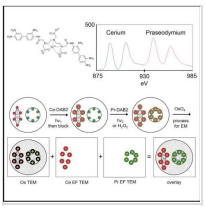

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Ln-DAB2 Solutions

Stephen R. Adams[1,2], Mason R. Mackey[2], Roger Tsien[1,3,4], Mark Ellisman[2,5], Jeffrey D. Martell[6]

[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]Howard Hughes Medical Institute, University of California, San Diego, La Jolla CA;
[4]Department of Chemistry & Biochemistry, University of California, San Diego, La Jolla CA;
[5]Department of Neurosciences, University of California, San Diego, La Jolla CA;
[6]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

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NCMIR@UCSD

NCMIR
University of California, San Diego

ABSTRACT

We use multicolor EM (electron microscopy) to paint multiple cellular markers by locally depositing specific Ln3+ from prepared solutions of Ln-DAB2 by mSOG, APEX2 or HRP. Each Ln3+ is then visualized by electron energy-loss spectroscopy and energy-filtered EM. Elemental maps are overlaid on conventional EM give multicolor EM.

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GUIDELINES

Ln-DAB2 solutions in cacodylate buffer were prepared immediately before use at room temperature.

Note: This buffer is hazardous and any waste should be handled accordingly.

SAFETY WARNINGS

Wear PPE.

1 To make 10 mL of a 2 mM Ln, Ce or Pr-DAB$_2$ solution, 15.6 mg (20 µmol) of DTPA-DAB$_2$ is suspended in 0.25 mL N,N Dimethylformamide (DMF) and gently heated to about 50C and

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sonicated/vortexed to dissolve.

2 8.33 mL of DDH2O is added to give a cloudy solution that cleared on addition of $LnCl_3$ aqueous solution (0.1 M of $LaCl_3 \cdot 6H_2O$, $CeCl_3 \cdot 6H_2O$, or $PrCl_3 \cdot xH_2O$; the latter stock solution is dissolved in 0.1 M HCl) with 120 µL of La or Ce solutions or 140 µL of Pr solution, followed by vortexing and bath sonication to give clear light-brown solutions.

3 Aqueous NaOH solution (1 M) is added sequentially in six equal portions (6 × 10 µL) with vortexing after each addition.

(A precipitate is initially formed during the early steps of this neutralization but a mostly clear solution is present by the end).

4 1.67 mL of 0.3 M sodium cacodylate buffer, pH 7.4 is added, mixed, and centrifuged (3000 × g, 10 min) to remove any precipitate.

5 Solutions are syringe-filtered (0.22 µm, Millipore) immediately prior to addition to cells.

6 Metal ion concentrations can be measured by inductively coupled plasma mass spectroscopy (Agilent 7700).

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