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DAB precipitation by Laccase Oxidation

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Song-Yi Lee[1], Heegwang Roh[1], Mason Mackey[2], Alice Y. Ting[1], Mark Ellisman[2]
[1]Stanford University; [2]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

Genetically-encoded oxidase, LaccID, oxidizes and precipitates DAB with oxygen and therefore without the need for H2O2 or light. LaccID-expressing HEX293T cells precipitated DAB and were processed for TEM.

Materials

1. MatTek dishes containing 35mm glass bottom No. 0 coverslips coated with poly-d-lysine (P35GC-0-14C, MatTek Corporation)

2. 0.1M sodium cacodylate with 2 mM $CaCl_2$, pH 7.4 (12310, Electron Microscopy Sciences)

3. 2% glutaraldehyde (18426, Ted Pella Incorporated)

4. 3,3′- Diaminobenzidine (DAB) (D8001-10G, Sigma-Aldrich)

5. 1% osmium tetroxide (EMS, 19150)

6. Ethanol series

7. Durcupan ACM resin (Sigma-Aldrich, 44610)

Safety warnings

Use PPE for toxic chemicals.

Cell Fixation

1 LaccID-expressing HEX293T cells are plated onto MatTek dishes containing 35mm glass bottom No. 0 coverslips coated with poly-d-lysine (P35GC-0-14C, MatTek Corporation).

2 Cells are fixed using warm 2% glutaraldehyde (18426, Ted Pella Incorporated) in buffer 0.1M sodium cacodylate with 2 mM $CaCl_2$, pH 7.4 (12310, Electron Microscopy Sciences) and then quickly moved to ice for 55 minutes.

3 Cells are washed five times for 2 min each on ice with cold 0.1M sodium cacodylate with 2 mM $CaCl_2$, pH 7.4

4 Cell are blocked for 5 min with cold 20 mM glycine in 0.1M sodium cacodylate with 2 mM $CaCl_2$, pH 7.4

5 A freshly solution of 5.4 mg of 3,3′- Diaminobenzidine (DAB) (D8001-10G, Sigma-Aldrich) is dissolved in 1.0 ml of 0.1 N HCl and added to 9.0 ml 0.1M sodium cacodylate buffer with 2 mM $CaCl_2$, pH 7.4 buffer.

6 The DAB solution is added to cells for at least 3-30 min at room temperature until the desired brown intensity color of the precipitate was visible.

7 Cells are then washed five times for 2 min each with cold 0.1M sodium cacodylate with 2 mM $CaCl_2$, pH 7.4.

TEM processing

8 Cells are post-fixed with 1% osmium tetroxide (EMS, 19150) in 0.1 M sodium cacodylate buffer pH 7.4 for 30 min on ice.

9 Postfixative is removed and cells are washed (five times for 1 min each) with 0.1 M sodium cacodylate buffer pH 7.4 containing 2 mM $CaCl_2$ on ice

10 Cells are washed (five times for 1 min each) with cold double-distilled H_2O (ddH_2O) on ice.

11 Cells are dehydrated with an ethanol series (20%, 50%, 70%, 90% and 100%) on ice for 1 min each.

12 Cells are dehydrated with 100% dry ethanol at room temperature three times for 1 min each.

13 Cells are infiltrated with 50:50 dry ethanol:Durcupan ACM resin (Sigma-Aldrich, 44610) for 30 min and then four changes of Durcupan at 1 h each and finally embedded in a vacuum oven at 60 °C for 72 hours.

Protocol references

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1. **Department of Genetics, Stanford University, Stanford, CA, USA**
   Song-Yi Lee, Daniel Hoces, Chang Lin, Rogelio A. Hernández-López & Alice Y. Ting
2. **Department of New Biology, DGIST, Daegu, Republic of Korea**
   Song-Yi Lee
3. **New Biology Research Center, DGIST, Daegu, Republic of Korea**
   Song-Yi Lee
4. **Department of Chemistry, Stanford University, Stanford, CA, USA**
   Heegwang Roh & Alice Y. Ting
5. **Department of Biology, Stanford University, Stanford, CA, USA**
   Alice Y. Ting
6. **The Phil & Penny Knight Initiative for Brain Resilience at the Wu Tsai Neurosciences Institute, Stanford, CA, USA**
   Alice Y. Ting
7. **Department of Neurosciences, University of California, San Diego, La Jolla, CA, USA**
   Mason R. Mackey, Keun-Young Kim & Mark H. Ellisman
8. **Center for Research in Biological Systems and the National Center for Microscopy and Imaging Research, University of California, San Diego, La Jolla, CA, USA**
   Mason R. Mackey & Mark H. Ellisman
3. **Department of Pharmacology, University of California, San Diego, La Jolla, CA, USA**
   Stephen Adams