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DAB precipitation by Laccase Oxidation

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We use this protocol and it's working

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Abstract

Genetically-encoded oxidase, LacclD, oxidizes and precipitates DAB with oxygen and therefore without the need for H₂O₂ or light. LacclD-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 CaCl₂, 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 Cells 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 0.1M 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 0.1M 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 (dd H_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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