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Protocol status: Working
We use this protocol and it's working

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DAB Staining

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ASAP Collaborative Research Network

Kaplitt Protocols

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ABSTRACT

3, 3'-diaminobenzidine (DAB) staining of mouse brain tissue

GUIDELINES

All steps should be done on an orbital shaker.

MATERIALS

Antibodies:

- anti-Tyrosine Hydroxylase (ab152), dilution 1:500
- Biotin-conjugated goat anti rabbit secondary antibody, dilution 1:300

Reagents:

- 1x PBS
- 30% H202
- Methanol
- ABC kit: Vector labs https://vectorlabs.com/vectastain-elite-abc-kit-standard.html
- Primary TH: AB 152, Millipore, NFAB152
- Secondary: anti-rabbit lgG (whole molecule) Biotin Conjugate, Sigma B-6648
- DPX mounting medium, Sigma Aldrich, https://www.sigmaaldrich.com/cata1og/product/SIGMA/06522?lang=en®ion=SE
- Antigen retrieval: Tris-EDTA, pH 9.0 or Sodium citrate, pH=6.0, 80°
- Tris-EDTA buffer (lOmM Tris Base, 1 mM EDTA, 0.05% Tween 20, pH 8.0): Mix 1.21 g Tris Base, 0.37 g of disodium EDTA in 1000 ml of distilled water. Adjust the pH to 9.0 with 1N Na OH and then add 0.5 ml of Tween 20. Store at RT for up to 3 months; for extended storage, store at 4 °C.
- Sodium citrate buffer: Tri-sodium citrate (dihydrate) 2.94 g, Distilled water 1 L. Mix to dissolve. Adjust pH to 6.0 with 1N HCI. Add 0.5 ml Tween 20 and mix well. Store at RT for 3 months or at 4°C for longer storage.

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SAFETY WARNINGS

The DAB solution is highly toxic and should be discarded properly according to institutional guidelines.

Staining/Mounting Process

1 Wash freshly sectioned slices of tissue 2-3 times with 1X PBS to remove OCT.

2 Quench sections for 00:15:00 in 3 mL of quenching solution (0.1 mL 30% H_2O_2, 0.1 mL methanol, and 0.8 mL 1X PBS). 15m

3 Wash 4-5 times in 1X PBS.

4 Block sections in 2 mL of 5% goat serum in 0.25% T-PBS for 01:00:00 1h Room temperature .

5 Stain with primary antibody (pSer129 primary antibody 1:500) overnight at 4 °C . Dilute primary antibody in 2.5% serum in 0.25% T-PBS (1 mL per well).

6 Wash sections 4-5 times with 1X PBS.

7 Transferred sections into secondary solution (1 mL of 1% serum in 0.25% T-PBS) for 2h 02:00:00 at Room temperature .

8 Wash sections 4-5 times in 1X PBS.

9 Transfer into ABC Kit solution (PK4000, Vector Laboratories) containing 10µl of solution A and 10µl of solution B for 1ml of 1X PBS for 1h 01:00:00 at Room temperature .

10 Wash sections 4-5 times in 1X PBS.

11 Transfer sections to the DAB working solution (SK-4100, Vector Laboratories) under a fume hood. Monitor until it stains well. Well-plate containing sections were gently shaken during staining and the reaction was stopped with transfer to 1X PBS based on dark-signal intensity on the fastest arising staining.

12 Wash sections 3-5 times in 1X PBS.

13 Mount sections on microscope slides.

14 Dry sections Overnight at Room temperature . 1h

15 Dehydrate sections starting with 2 baths of distilled water (~ 00:02:00). 2m

16 Wash with 70% ethanol (2 times ~ 00:02:00). 2m

17 Wash with 95% ethanol (2 times ~ 00:02:00). 2m

18 Wash with 100% ethanol (2 times ~ 00:02:00). 2m

19 Wash with 100% xylene (2 times ~ 00:05:00) to allow section dehydration. 5m

20 Dry sections at Room temperature (~ 00:05:00) and covered with DPX mounting medium. 5m