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DAB Staining of Fixed Mouse Brain Tissue Sections

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We use this protocol and it's working

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Abstract

This protocol describes steps for immunohistochemical 1 3,3′-Diaminobenzidine (DAB) staining of free floating fixed mouse brain tissue sections.

Materials

- 24-well netwell insert (Fisher NC9979302)
- dPBS (UCSF Stem Cell Core)
- Tris buffered saline (TBS)
- Methanol
- 30% H_2O_2 (Millipore Sigma H1009)
- Endogenous peroxidase quenching buffer:
  - 10% MeOH, 3% H_2O_2, in 1X TBS
- TBS++++ blocking buffer:
  - 10mL 10X TBS
  - 10mL fetal bovine serum (UCSF Stem Cell Core)
  - 3g BSA (Millipore Sigma A3803)
  - 1g glycine
  - 30mg sodium azide (Millipore Sigma S2002)
  - Bring to 96mL with ddH_2O
  - Store @-20˚C in 12mL aliquots
  - Add 500 µL of 10% Triton x-100 (Millipore Sigma 93443) right before use
- Primary antibody (e.g. rabbit anti-TH, Abcam AB152)
- ABC complex - VECTASTAIN® Elite ABC-HRP Kit, Peroxidase (Rabbit IgG)
  - Manufacturer: Vector Laboratories
  - Cat#: PK-6101
  - Includes biotinylated goat anti-rabbit IgG secondary antibody (Vector Laboratories BA-1000-1.5)
- 50X 1 3,3'-diaminobenzidine (DAB) dissolved in 0.1M Tris
  - Millipore Sigma D12384
- 0.1 M Tris, pH 8.0
- Ethanol
- Xylene (ThermoFisher Scientific X51)
- Permount mounting medium (ThermoFisher Scientific SP15)
- Superfrost Plus slides (Fisher 12 550 15)

Troubleshooting

Day 1

1 Blocking and primary antibody incubation.

1.1 Pull all sections into 1x dPBS in 24-well netwells.

1.2 Wash 3 times with 1x dPBS, 10 mins each.

1.3 Wash 3 times with 1x TBS, 10 mins each.

1.4 Quench endogenous peroxidase activity with quenching buffer, 5 mins at RT. Just covering the sections is sufficient, and drain excess buffer by dabbing on kimwipes before and after.

1.5 Wash 3 times with 1x TBS, 10 mins each.

1.6 Block in TBS++++ for 75 mins at RT on rocker, 2 mL/well.

1.7 Incubate in primary antibody diluted in TBS++++ on rocker at RT O/N, 500 µL/well in a new 24-well plate without netwell insert.

Day 2

2 Secondary antibody incubation and DAB development

2.1 Wash 3 times with 1x TBS, 5 mins each in netwell insert.

2.2 Incubate in secondary antibody at a 1:300 dilution in TBS++++ for 2 hrs, 500 µL/well in a 24-well plate without netwell insert.

2.3 15 mins prior to end of incubation, prepare ABC complex:
- Both buffer A and B are 1:300 dilution in TBS++++

- Incubate in RT beaker of water for 30 mins, in drawer

2.4 Wash 3 times with 1x TBS, 5 mins each in netwell insert.

2.5 Incubate 2 hours in ABC complex in TBS++++ at RT on rocker without netwell insert.

2.6 Wash 3 times with 0.1 M Tris buffer, pH 8.0, 5 mins each in netwell insert.

2.7 Prepare 1x DAB solution:
- Thaw aliquots of 50x DAB, 1 tube is around 200 µL
- Add 50x DAB into 0.1M Tris buffer
- Vortex for 5 sec to mix well, sit in dark for 10 mins
- Right before development, add 30% H2O2 (1:10000 dilution), vortex to mix well
- Distribute 2 mL to each well on a 12-well plate

2.8 Prepare an additional 12-well plate with Tris buffer to start washes immediately after development.

2.9 Develop DAB reaction, minimizing time difference between wells. Drain excess buffer by dabbing on kimwipes before and after.

2.10 Wash 2 times with Tris, 5 mins each.

2.11 Wash 2 times with TBS, 5 mins each.

2.12 Wash 2 times with PBS, 10 mins each.

2.13 Sections can be stored in PBS at 4C.

2.14 Mount onto Superfrost Plus slides labeled with pencil and allow to dry completely.

Day 3

3 Dehydration and clearing.

3.1 Wash 2 × 2min in ddH2O.

3.2 Wash 2 × 2min in 70% EtOH.

3.3 Wash 2 × 2min in 95% EtOH.

3.4 Wash 2 × 2min in 100% EtOH.

3.5 Wash 2 × 2min in Xylene.

3.6 Coverslip using Permount mounting medium.

3.7 Let dry in fume hood overnight then store in drawer in slide box until imaging.