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Free Floating DAB Staining

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

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Abstract

This protocol describes the procedure for performing 3,3'-Diaminobenzidine (DAB) immunohistochemical staining on free-floating tissue sections. The method is optimized to preserve tissue integrity and enhance antibody penetration by processing sections without being mounted to a slide. It results in a stable, permanent chromogenic precipitate for the microscopic visualization and analysis of antigen distribution.

Safety warnings

Be sure to do DAB developing in a fume hood and dispose of all apparatus that touched DAB solution in bucket of bleach and water.

DAY 1

1 Wash sections 3×5min in TBS

2 Quench sections with solution for 5min at RT
Quenching solution (10mL – 3% H2O2 in 1XTBS):
4.5mL TBS
4.5mL MeOH
1mL 30% H2O2

3 Wash 2×5min TBS

4 Put sections in antigen retrieval for 30mins at 37C with agitation
Antigen retrieval in TBS:
10mM sodium citrate
0.05% Tween-20

5 Wash 3×5min in TBS

6 Block in 5% serum + TBST for at least 1hr @ room temp

7 24-well plate (500µL/well): dilute primary Ab in TBST + 1% serum (of secondary host animal).

8 Incubate overnight at 4C

Day 2

9 Wash sections 3×10min in TBST

10 Incubate sections with diluted biotinylated secondary Ab [(1:1000) in TBST + 1% serum] for 2hrs at RT in the dark

11 Wash sections 3×10min in TBS in the dark

12 Dilute ABC solution (1:5 in TBS) and incubate sections for 30min at RT (using 2mL/well)

13 Wash 3×10mins in TBS

14 Follow DAB kit instructions (work in the hood, set up a bucket with bleach and water)

15 Prepare substrate working solution. To every 5mL of diH20, add:

16 2 drops of Buffer Stock Solution

17 4 drops of DAB Stock Solution

18 2 drops of Hydrogen Peroxide Solution

19 If gray-black reaction is desired, add 2 drops of Nickel Solution

20 Move samples to substrate solutions and develop until sufficient colour change is evident; around 3-6mins

21 Immediately wash 3×5min in TBS

22 Mount sections on slides and dry for approximately 30min to 1hr or just until sections are adhered to slide.

23 Dehydrate:
70% ethanol 2×3min
90% ethanol 2×3min
95% ethanol 2×3min
100% ethanol 2×3min
Citrisolve 2×3min

24 Using Permount, cover with coverslip and dry several hours up to 2 days @RT.