Aug 23, 2025

Free Floating DAB Staining

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.q26g7pxqqgwz/v1

Ashley Harms[1], Jhodi Webster[1]

[1]University of Alabama at Birmingham

Jhodi Webster
University of Alabama at Birmingham

DOI: https://dx.doi.org/10.17504/protocols.io.q26g7pxqqgwz/v1

Protocol Citation: Ashley Harms, Jhodi Webster 2025. Free Floating DAB Staining. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7pxqqgwz/v1

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

Created: October 14, 2023

Last Modified: August 23, 2025

Protocol Integer ID: 89279

Keywords: ASAPCRN, immunohistochemical staining, floating tissue section, floating dab, antibody penetration by processing section, permanent chromogenic precipitate for the microscopic visualization, antibody penetration, antigen distribution, analysis of antigen distribution, tissue section, dab, tissue integrity, permanent chromogenic precipitate, microscopic visualization

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.