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

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Immunohistochemistry Protocol for Paraffin-Embedded Sections - General

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ABSTRACT

This protocol details general immunohistochemistry procedure.

ATTACHMENTS

[gegpbqbhp.pdf](#)

MATERIALS

For DAB:

Solution-based: Sigma Cat# D5637; prepared in Tris buffer as per manufacturer's instructions

SIGMAFAST DAB Tablets: Sigma Cat# 4293

SIGMAFAST DAB with Metal Enhancer - Sigma Cat# 0426

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Immunohistochemistry
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Day 1 - Deparaffinize

1 Dip slides in 100% Citrisolv 3 x 5 mins.

1.1 Dip slides in 100% Citrisolv 3 x  00:05:00 (1/3).

5m

1.2 Dip slides in 100% Citrisolv 3 x  00:05:00 (2/3).


5m

1.3 Dip slides in 100% Citrisolv 3 x  00:05:00 (3/3).


5m

Hydration

2 Dip in 100% EtOH 2 x 2 mins.


2.1 Dip in 100% EtOH 2 x  00:02:00 (1/2).

2m


2.2 Dip in 100% EtOH 2 x  00:02:00 (2/2).

2m

3 Dip in 95% EtOH 2 x 2 mins.


3.1 Dip in 95% EtOH 2 x  00:02:00 (1/2).

2m


3.2 Dip in 95% EtOH 2 x  00:02:00 (2/2).

2m

4 Dip in 80% EtOH 2 x 2 mins.


4.1 Dip in 80% EtOH 2 x  00:02:00 (1/2).

2m


4.2 Dip in 80% EtOH 2 x  00:02:00 (2/2).

2m

5 Dip in ddH₂O 2 x 2 mins.

5.1 Dip in ddH₂O 2 x  00:02:00 (1/2).

2m

5.2 Dip in ddH₂O 2 x  00:02:00 (2/2).

2m

Quench endogenous peroxides

6 Incubate slides in 0.3% H₂O₂ in methanol for  00:15:00 ( 500 µL of 30% H₂O₂ in  50 mL MetOH, in white containers).

15m







Antigen retrieval


7

5m

Note

Use citrate buffer  6 or Tris-EDTA buffer  9 based on antigen desired.

Pre-heat antigen retrieval solution in microwave in white containers (~  50 mL), for  00:05:00 at full power. Use large plastic container and fill halfway with water.

8 Transfer slides to white containers with AR solution. Microwave for  00:10:00 at power level 1.

10m

9 Rest slides at  Room temperature for  00:20:00 , change water in bottom of container.

20m

Wash

10 Remove AR solution and wash slides with ddH₂O for 00:05:00 . 5m



11 Draw bubble with hydrophobic pen during this wash.



12 Rinse slides with PBS for 00:05:00 . 5m



Blocking

13 Block with 5% normal goat serum in PBS + 0.1% Triton-x-100 + 0.05% Tween-20, 00:30:00 @ 30m
Room temperature (300 µL /slide).

Primary antibody


14 Dilute antibody in 5% normal goat serum in PBS + 0.1% Triton-x-100 + 0.05% Tween-20, Overnight 5m
at 4 °C (300 µL /slide).

Day 2 - Wash


15 Rinse with PBS; 3 x 5 minutes



15.1 Rinse with PBS; 3 x 00:05:00 (1/3). 5m


15.2 Rinse with PBS; 3 x  00:05:00 (2/3).

5m

15.3 Rinse with PBS; 3 x  00:05:00 (3/3).


5m

Secondary antibody

16 Add secondary antibody diluted 1:225 in PBS + 0.1% triton-X + 0.05% tween-20 with 5% normal goat serum (~  300 μ L /slide).



16.1 Commonly: biotinylated anti-rabbit IgG (H+L) made in goat (Vector Labs,BA-1000) or biotinylated anti mouse IgG (H+L) made in goat (Vector Labs, BA-9200).

17 Incubate at  Room temperature for  01:00:00 -  02:00:00 .

3h




Wash

18 Rinse with PBS; 3 x 5 minutes.




18.1 Rinse slides with PBS; 3 x  00:05:00 (1/3).

5m

18.2 Rinse slides with PBS; 3 x  00:05:00 (2/3).

5m

18.3 Rinse slides with PBS; 3 x  00:05:00 (3/3).




5m




Amplification

19 Prepare amplification solution at least  00:30:00 before use.

30m

20 Amplification with VECTASTAIN® Elite® ABC HRP Kit.

20.1  9 µL solution A per mL PBS, vortex; +  9 µL solution B per mL PBS, vortex; ( 300 µL /slide).


20.2 Incubate at  Room temperature for  01:00:00 -  02:00:00 .

3h




Wash


21 Rinse slides with PBS; 3 x 5 minutes

21.1 Rinse slides with PBS; 3 x  00:05:00 (1/3).

5m

21.2 Rinse slides with PBS; 3 x  00:05:00 (2/3).

5m

21.3 Rinse slides with PBS; 3 x  00:05:00 (3/3).

5m

Substrate development

22

Note

Use DAB or eDAB depending on antigen being detected.

For regular DAB:


22.1 Add  1 mL of DAB to  150 mL of [M] 50 millimolar (mM) Tris +  50 μ L 30% H₂O₂.



22.2 Dip slides into DAB solution, develop until colour appears ~  00:03:00 .

3m

23 For eDAB (SIGMAFAST™ DAB with Metal Enhancer):

23.1 Add 1 tablet DAB/Cobalt and 1 tablet Urea Hydrogen Peroxide to  5 mL ddH₂O, vortex until dissolved.



Note

Prepare immediately before use.


23.2 Use ~  300 μ L /slide, develop until colour appears ~  00:03:00 .

3m



24 Rinse with PBS to stop reaction.



25 Rinse with ddH₂O for  00:05:00 .

5m




Counterstain

26 Counterstain with 1:2 Hematoxylin:ddH₂O, dip slides in quickly.






27 Remove excess hematoxylin in ddH₂O; 2X.

Dehydration

28 5 dips, 1 minute each:

28.1 Dip in 50% EtOH  00:01:00

1m

- 28.2** Dip in 80% EtOH x2  00:01:00 1m
- 28.3** Dip in 95% EtOH  00:01:00 1m
- 28.4** Dip in 100% EtOH x2  00:01:00 1m
- 28.5** Dip in 1:1 Citrisolv: 100% EtOH  00:01:00 1m
- 28.6** Dip in 100% Citrisolv x2  00:01:00 1m

Coverslip

- 29** Coverslip with permount solution, allow slides to dry before viewing on microscope.