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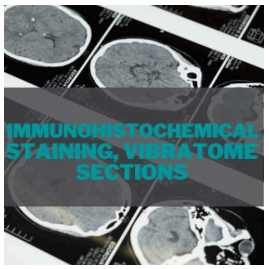
Immunohistochemical staining, vibratome sections



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

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Abstract

This protocol outlines general procedures for Immunohistochemical staining, using vibratome sections.

Attachments

PDF

Immunohystochemical_..

139KB

Materials

Reagents Needed

- 3% H_2O_2 in 1 x PBS
- 1 x PBS 0.1% Triton X-100
- 1 x PBS 0.1% Triton X-100 + 10% serum (accordingly to the secondary Abs)
- Primary Antibodies (TH Ab152 1/5000)
- Biotinylated Secondary Antibodies (SAR 1/300)
- Streptavidine-HRP complex (1/1000)
- DAB + H_2O_2 (1.4 µl H_2O_2 for 5 ml filtered DAB solution)
    - DAB solution: 10 mg DAB (=1 tablet) for 25 ml 0.05 TRIS (TBS) pH 7.6; dissolve and filter through 0.22 µm filter, add H_2O_2 just before
- PBS or PBS + 0.1% Na Azide
- ½ PBS + ½ AD
- Ethanol:
    - 70% ethanol
    - 90% ethanol
    - 100% ethanol
- Histoclear II
- DPX mountant

Consumables Needed

- Coverslips
- Slides

Equipment Needed

- Wobbler

Safety warnings

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Before start

This protocol uses floating sections in 24-well plate in `[M] 1 X PBS`.

Quench Endogenous Peroxidase Activity

20m

1 Incubate samples in 1 x PBS 3% H_2O_2, for 00:10:00 at Room temperature on the wobbler (to quench endogenous peroxidase activity) using 500 µL / well. 10m

2 Rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS .

3 Rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS for 00:05:00 on wobbler. 5m

4 Repeat: rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS for 00:05:00 on wobbler. 5m

Primary Antibody Incubation

40m

5 Add 250 µL Primary Abs in 1 x PBS 0.1% Triton X-100 + 10% serum (accordingly to the secondary Abs) Overnight at Room temperature on wobbler. 40m

> Note
>
> Dilution: 1st AB: TH Ab152 1/5000

6 Rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS .

7 Rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS for 00:05:00 on wobbler. 5m

8 Repeat: rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS for 00:05:00 on wobbler. 5m

Secondary Antibody Incubation

20m

9 Add 250 µL biotinylated Secondary Abs in 1 x PBS 0.1% Triton X-100 for 00:30:00 at Room temperature on wobbler. 30m

Note

Dilution: 2nd AB: SAR 1/300

10 Rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS .

11 Rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS for 00:05:00 on wobbler. 5m

12 Repeat: rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS for 00:05:00 on wobbler. 5m

Streptavidine-HRP complex Incubation 30m

13 Add 250 µL Streptavidine-HRP complex 1/1000 (in 1 x PBS 0.1% Triton X-100) for 00:30:00 at Room temperature on wobbler. 30m

Note

Dilution: STRP-HRP 1/1000

14 Rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS .

15 Rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS for 00:05:00 on wobbler. 5m

16 Repeat: rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS for 00:05:00 on wobbler. 5m

DAB + H_2O_2

17

Safety information

Work on DAB Bench!

Prepare DAB solution: 10 mg DAB (=1 tablet) for 25 mL 0.05 TRIS (TBS) pH 7.6 ; dissolve and filter through 0.22 µm filter, add H_2O_2 just before use! Or Vector SG (TH). (Add 1.4 µL H2O2 for 5 mL filtered DAB solution).

18 Add 250 µL DAB + H2O2 ***(ON DAB BENCH!)***. Allow reaction to proceed for a few minutes at Room temperature **without wobbling**.

19 Rinse with [M] 0.1 % (v/v) Triton X-100 in 1x PBS .

20 Replace TBS Triton X-100 with PBS or [M] 0.1 % (v/v) Sodium Azide in PBS in case sections are not to be mounted immediately. Store at 4 °C .

½ PBS + ½ AD Rinse

30m

21 Briefly rinse sections in ½ PBS + ½ AD and mount on gelatin coated microscopy slides. Allow to dry for 00:30:00 in flow or couple of hours on bench. 30m

Dehydration

25m

22 Dehydrate samples in [M] 70 % (v/v) ethanol for 00:05:00 . 5m

23 Dehydrate samples in [M] 90 % (v/v) ethanol for 00:05:00 . 5m

24 Dehydrate samples in [M] 100 % (v/v) ethanol for 00:05:00 . 5m

25 Repeat: dehydrate samples in [M] 100 % (v/v) ethanol for 00:05:00 . 5m

26 Replace ethanol with Histoclear II for 00:05:00 . 5m

Mounting 30m

27 Mount coverslips on top of the slides with DPX and allow to dry Overnight in the flow. 30m

28 Press out bubbles next morning.