NeuN Immunohistochemistry Protocol Version 2

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Abstract

Protocol for Immunohistochemistry free-floating sections with anti-NeuN antibody for avian tissue.

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Materials

- Boric acid View by P212121
- Ammonium Chloride View by P212121
- Sodium Acetate, Trihydrate by Contributed by users
- Sodium Phosphate monobasic by Contributed by users
- Triton X-100 T8787 by Sigma Aldrich
- Sodium phosphate dibasic S3264 by Sigma Aldrich
- VECTASTAIN Elite ABC HRP Kit (Peroxidase, Standard) PK-6100 by Vector Laboratories
- Ammonium nickel(II) sulfate hexahydrate 574988 by Sigma Aldrich
- 3,3′-Diaminobenzidine tetrahydrochloride D5905 by Sigma Aldrich
- α-D-Glucose 158968 by Sigma Aldrich
- Normal Goat Serum Blocking Solution S-1000 by Vector Laboratories
- Anti-NeuN Antibody, clone A60 MAB377 by Merck Millipore
- Biotinylated Goat Anti-Mouse IgG Antibody BA-9200 by Vector Laboratories
- PBS by Contributed by users

Protocol

Antigen Retrieval Method

Step 1.

To remove the excess paraformaldehyde fixative, wash with 0,1 M PBS the sections free-floating at agitation in room temperature for 3 min. (1/3)

DURATION

00:03:00

Antigen Retrieval Method

Step 2.

To remove the excess paraformaldehyde fixative, wash with 0,1 M PBS the sections free-floating at agitation in room temperature for 3 min. (2/3)

DURATION

00:03:00

Antigen Retrieval Method

Step 3.

To remove the excess paraformaldehyde fixative, wash with 0,1 M PBS the sections free-floating at agitation in room temperature for 3 min. (3/3)

DURATION

00:03:00

Antigen Retrieval Method

Step 4.

Incubate with 12% Boric acid (pH =9,0, 70°C) in a water bath for 1 hour.

DURATION

01:00:00

NOTES

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When the temperature reaches 50°C, using a brush put the sections at the recipient and wait the temperature reaches 70°C and keep at this temperature for 1 hour.

Antigen Retrieval Method

Step 5.

Remove the recipients from water bath and wait until it is at room temperature.

Antigen Retrieval Method

Step 6.

Wash the sections with 0,1% PBS/T for 5 minutes. (1/3)

DURATION

00:05:00

Antigen Retrieval Method

Step 7.

Wash the sections with 0,1% PBS/T for 5 minutes. (2/3)

DURATION

00:05:00

Antigen Retrieval Method

Step 8.

Wash the sections with 0,1% PBS/T for 5 minutes. (3/3)

DURATION
00:05:00

Antigen Retrieval Method

Step 9.

Wash the sections with 0,1M PBS for 2 minutes (shaking). (1/3)

DURATION
00:02:00

Antigen Retrieval Method

Step 10.

Wash the sections with 0,1M PBS for 2 minutes (shaking). (2/3)

DURATION
00:02:00

Antigen Retrieval Method

Step 11.

Wash the sections with 0,1M PBS for 2 minutes (shaking). (3/3)

DURATION
00:02:00

Protein Blocking Step

Step 12.

Incubate with blocking buffer (Normal Goat Serum Blocking Solution S-1000 10% in 0,3% PBST) for 12 hours at gentle agitation in refrigeration 4°C.

DURATION
12:00:00

Primary Antibody

Step 13.

Remove the serum and incubate with Anti NeuN Antibody (MAB377 Anti-NeuN Antibody, clone A60), diluted in 0,3%PBS/T at gentle agitation, overnight at 4°C.

Primary Antibody

Step 14.

Wash the sections with 0,1M PBS/T 0,1% for 2 minutes (shaking). (1/3)

DURATION
00:02:00

Primary Antibody

Step 15.

Wash the sections with 0,1M PBS/T 0,1% for 2 minutes (shaking). (2/3)

DURATION
00:02:00

Primary Antibody

Step 16.

Wash the sections with 0,1M PBS/T 0,1% for 2 minutes (shaking). (3/3)

DURATION
00:02:00

Secondary Antibody

Step 17.

Incubate with secondary antibody (Biotinylated Goat Anti-Mouse IgG Antibody, BA-9200, Vector Laboratories) diluted in 0,3% PBS/T at 1:250, for 1 hour at room temperature.

DURATION
01:00:00

Blocking Step

Step 18.

Incubate with 0,3% hydrogen peroxide (diluted in 0,1 M PBS) for 15 minutes with light shaking.

DURATION
00:15:00

Blocking Step

Step 19.

Wash sections with PBS/T 0,1% for 2 minutes (shaking).(1/3)

DURATION
00:02:00

Blocking Step

Step 20.

Wash sections with PBS/T 0,1% for 2 minutes (shaking).(2/3)

DURATION
00:02:00

Blocking Step

Step 21.

Wash sections with PBS/T 0,1% for 2 minutes (shaking).(3/3)

ABC

Step 22.

Incubate in VECTASTAIN® ABC KIT solution for 1 hour (total) at 4ºC with light shaking.

First 37,5 µl A + 37,5µl B with 1,88 ml 0,3%PBS/T for 30 minutes.

DURATION
00:30:00

ABC

Step 23.

Add 13,12 ml 0,3%PBS/T and incubate for 30 more minutes at 4ºC with light shaking.

DURATION
00:30:00

ABC

Step 24.

Wash with 0,1% PBS /T for 5 minutes, (shaking). (1/2)

DURATION
00:05:00

ABC

Step 25.

Wash with 0,1% PBS /T for 5 minutes, (shaking). (2/2)

DURATION
00:05:00

DAB Visualization-GDN preparation

Step 26.

Firstly prepare the Solution A by mix 0,006g of Diaminobenzidine (DAB) with 5 ml of distilled water.

DAB Visualization-GDN preparation

Step 27.

Secondly, prepare the Solution B by mixing 0,250g of Nickel ammonium sulfate with 5 ml de Acetate Buffer pH 6.0.

DAB Visualization-GDN preparation

Step 28.

Thirdly mix Solution A and B adding ammonium chloride (0,004g) with 0.020g α-D-Glucose.

DAB Visualization-GDN preparation

Step 29.

Leave the section in this mix of A and B for 5 minutes.

DAB Visualization

Step 30.

Incubate sections with solution GND and wait for 3 minutes.

DURATION
00:03:00

NOTES
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After each step remove the remaining solution from the previous step with a pipette, with careful not to damage or lose sections.

Use only sterilized material to minimize the risk of contamination between different antibodies or solutions.

At the end add chlorine to inactivate GND + DAB residual solution before disposal on an appropriate container.

DAB Visualization

Step 31.

Add 0,007g of Glucose-oxidase for each 3ml of GND solution for revelation. Stop revelation when the goal contrast is achieved (use a low gain microscope).

DAB Visualization

Step 32.

Remove the GDN + Glucose oxidase and wash the sections using 0,1M PBS for 2 minutes with light shaking. (1/3)

DURATION
00:02:00

DAB Visualization

Step 33.

Remove the GDN + Glucose oxidase and wash the sections using 0,1M PBS for 2 minutes with light shaking. (2/3)

DURATION
00:02:00

DAB Visualization

Step 34.

Remove the GDN + Glucose oxidase and wash the sections using 0,1M PBS for 2 minutes with light shaking. (3/3)

DURATION

00:02:00

DAB Visualization

Step 35.

Mount the sections in appropriate gelatinized microscope slides and dry at room temperature for 12 hours or more depending on the mounting medium of choice.

DURATION

12:00:00

DAB Visualization

Step 36.

Dihydrate and add the coverslips.