# NeuN Immunohistochemistry Protocol

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## Abstract

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

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## Protocol

### Antigen Retrieval Method

**Step 1.**

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, 3 times.
2. Incubate with 12% Boric acid (pH =9,0, 70°C) in water bath for 1 hour. 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. Remove the recipients from water bath and wait until it is at room temperature.
3. Wash the sections with 0,1% PBS/T for 5 minutes at , 3 times.
4. Wash the sections with 0,1M PBS for 2 minutes (shaking), 3 times each.

### Protein Blocking Step

**Step 2.**

1. 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.

### Primary Antibody

**Step 3.**

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

### Secondary Antibody

**Step 4.**

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

## Blocking Step

**Step 5.**

1. Incubate with 0,3% hydrogen peroxide (diluted in 0,1 M PBS) during 15 minutes with light shaking.
2. Wash sections with PBS/T 0,1% during 2 minutes, 3 times (shaking).

## ABC

**Step 6.**

1. Incubate in VECTASTAIN® ABC KIT solution (first 37,5 µl A + 37,5µl B with 1,88 ml 0,3%PBS/T for 30 minutes, after add 13,12 ml 0,3%PBS/T) during 1 hour at 4ºC with light shaking.
2. Wash with 0,1% PBS /T for 5 minutes, 2 times (shaking).

## DAB Visualization

**Step 7.**

1. GDN preparation

- Firstly prepare the Solution A by mix 0,006g of Diaminobenzidine (DAB) with 5 ml of distilled water.
- Secondly prepare the Solution B by mixing 0,250g of Nickel ammonium sulfate with 5 ml de Acetate Buffer pH 6.0
- Thirdly mix Solution A and B adding ammonium chloride (0,004g) with 0.020g α-D-Glucose.

Leave the section in this mix during 5 minutes.

2. Incubate sections with solution GND and wait for 3 minutes, after 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).
3. Remove the GDN + Glucose oxidase and wash the sections using 0,1M PBS for 3 times (2 minutes each time) with light shaking.
4. 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.
5. Dihydrate and add the cover slips.

REAGENTS

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 T8787-50ML by Sigma Aldrich

Sodium phosphate dibasic 7558-79-4 by Sigma Aldrich

VECTASTAIN Elite ABC HRP Kit (Peroxidase, Standard) PK-6100 by Vector Laboratories

Ammonium nickel(II) sulfate hexahydrate 7785-20-8 by Sigma Aldrich

3,3′-Diaminobenzidine tetrahydrochloride D5905 by Sigma Aldrich

α-D-Glucose 492-62-6 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

## NOTES

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

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

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