**NeuN Immunohistochemistry Protocol (frozen; updated December 2015)**

**Experimental info Reagent info (manufacturer, source)**

Researcher(s): 1° antibody: 1:600 Rabbit-anti-NeuN (Millipore, ABN78)

Experiment: 2° antibody: 1:500 AF555-conjugated Donkey-anti-rabbit (LifeTech, A31572)

Animal #(s): PBS (0.01M); PBT (0.01M PBS + 10% Triton X); DS (donkey serum)

**Procedure: Day 1 (date )**

1. Tissue fixation
   1. dry slides using a slide warmer
   2. fix tissue in NBF, 10 minutes, room temperature, gentle agitation; **start time**:
2. Washes
   1. PBS, 10 minutes, room temperature, gentle agitation; **start time**:
   2. PBT, 10 minutes, room temperature, gentle agitation; **start time**:
   3. PBT, 10 minutes, room temperature, gentle agitation; **start time**:
3. Non-specific tissue blocking
   1. 200 l /slide, 2 hours, room temperature, gentle agitation; **start time**: ;
      * **VDS: l** = Vtotal  l x 0.10 DS
      * **VTriton X: l** = Vtotal  l x 0.20 10% Triton X
      * **VPBS: l** = Vtotal l– VDS  l – VTriton X  l
4. 1° antibody incubation
   1. 200 l /slide, 1 hour, 37°C, *dark*, gentle agitation; **start time**: ;
      * **VDS: l** = Vtotal  l x 0.05 DS
      * **V1° Ab : l** = Vtotal  l x 0.0017 1° antibody
      * **VPBS: l** = Vtotal l– VDS  l – V1° Ab  l
   2. Overnight, 4°C, *dark*

**Procedure: Day 2 (date )**

1. Washes
   1. PBT, 10 minutes, room temperature; **start time**:
   2. PBT, 10 minutes, room temperature; **start time**:
   3. PBT, 10 minutes, room temperature; **start time**:
2. 2° antibody incubation
   1. 200 l /slide, 2 hours, room temperature; **start time**: ; 2° antibody calculation:
      * **VDS: l** = Vtotal  l x 0.03 DS
      * **VTriton X : l** = Vtotal  l x 0.003 Triton X
      * **V2° Ab : l** = Vtotal  l x 0.002 2° antibody
      * **VPBS: l** = Vtotal l– VDS  l – VTriton X  l – V2° Ab  l
3. Development
   1. Remove from 2° antibody, mount on slides, and apply Prolong Gold coverslips (*dark*)
   2. Incubate at room temperature for 24 hours
   3. Transfer to dark slide box and store at -20°C