A. Immunohistochemistry by avidin/biotin/peroxidase complex (ABC):

**Day one**

1. Deparaffinize sections in xylene, 5 min twice.
2. Rehydrate in a decreasing ethanol series: 100%, 95% x2, 70%, 50% 2 min each. Rinse in PBS, 3 min x 3.
3. Block endogenous peroxidases by incubation with 10% methanol, 3% H$_2$O$_2$ in PBS, 30 min at RT.
4. Rinse well in PBS: at least 4 changes, 5 min each.
5. Incubate slides in freshly prepared 0.02% pepsin (Sigma), 0.01 N HCl in PBS for 20 min at 37°C.
6. Denature DNA in 2 N HCl, for 45 min at RT.
7. Neutralize in 0.1 M sodium borate, pH 8.5, 10 min.
8. Wash with PBS for 10 min.
9. With a PAP pen, encircle the sections to be treated. Block non-specific interactions by incubating with 1 drop of horse serum in 3.3 ml PBS (about 1%) at RT for 30 min in a humidified chamber.
10. Incubate with mouse anti-BrdU IgG1 (Becton-Dickinson #7580) as the primary Ab at 1:20 in the blocking solution, in a humidified chamber, 4°C, O/N (about 5 hr minimum). As a control for Ab specificity, leave blocking solution alone on one slide, without antibody.

**Day two**

11. Allow slides to warm up at least 45 min at RT.
12. Aspirated off primary AB, and rinse in PBS 3 min x 5.
13. Incubate all slides with secondary Ab: 1 drop normal horse serum in 10 ml PBS, vortex, add 2 drops anti-mouse IgG-biotinylated Ab, vortex, and incubate for 1 hr at RT in a humidified chamber.
14. Approximately 45 min into the incubation, prepare avidin/biotin/peroxidase complex (ABC). Add 1 drop of A to 5 ml PBS, Vortex, ad 1 drop of B, vortex, and let sit at RT for at least 30 min.
15. Aspirate off secondary antibody and rinse in PBS, 3 min x 5.
16. Incubate sections in ABC, 30 min, at RT.
17. Aspirate off ABC, and rinse in PBS, 3 min x 3.
18. Incubate slides in pH 7.5 buffer, 15 min at RT.
19. Incubate with DAB solution: 10 mg DAB in 17 ml TNS (0.1m Tris-HCl, pH 7.5, 0.15m NaCl), add 17 µl H$_2$O$_2$. Block out the light to optimize the reaction, which usually takes 3-10 min.
20. PBS rinse when the reaction has stopped or background is getting high. After several minutes. Mount with aquamount. Store slides at 4°C.
21. Photograph under bright field.

Note: Do not let the sections dry between steps!
Note: all subsequent steps utilize VectaStain kits from Vector Laboratories. Blocking solutions, secondary Ab (horse anti-mouse IgG). And ABC reagents are from kit no.
Reagents for peroxidase reaction utilizing DAB/H₂O₂ as substrates are from kit no. SK-4100. The protocol is essentially that of the manufacturer, but some alterations suggested Dr. Jose Arnal (Noton Lab, AECOM Neurology) have been implemented.

Note: I have experienced degradation of the slide after about one month! Take photos ASAP!