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_2O_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 20min 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_2O_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.

PK-41002. Reagents for peroxidase reaction utilizing DAB/H_2O_2 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!