**Biotin-ABC-DAB staining Protocol by Jie**

1. Take slides from the freezer and thaw them for 30 min at RT
2. Rinse slides 3x for 10 minutes in 0.3% PBS-T while on shaker
3. Treat for 60 min in blocking solution
4. Dilute primary Ab Rabbit-a-CTB (1:500, Sigma C3062) in blocking buffer, and incubate the sections in primary Ab O/N
5. Rinse sections 3x for 10 minutes in 0.3% PBS-T while on shaker
6. Treat tissue sections in 3% H2O2 at RT for 10 mins.Dilute 1 part of 30%H2O2 solutin in 9 parts of Methonal)
7. Rinse sections 5 x for 10 minutes in 0.3% PBS-T while on shaker
8. Take Vectastain ABC kit out, use 100 ul Reagent A and 100 ul ReagentB, diluted in 10 ml of 1x PBST. Incubated the section in it for 2 hours @ RT.
9. Rinse sections 5 x for 10 minutes in 0.3% PBS-T while on shaker
10. Prepare DAB developing solution: Take one tablet of DAB and one tablet of H2O2, and dissolve them in 5 ml distilled water and vortex.Prepare freshly each time and use it within 1 hour. Don’t touch the tablets.
11. Incubate in the DAB-H2O2 developing solution for 1-5 mins. Incubation time varies based on the signal of biotin in your samples. Check the color every minute.
12. Rinse sections 5 x for 10 minutes in 0.3% PBS-T while on shaker.
13. Mount sections onto gelatin-coated slides, and dehydrate for 1 hour or overnight @RT.
14. Dehydrate through 50%, 70%, 80%, 90%, 95%, 100%-I, 100%-II alcohol for 5 mins each, then through Citrosol 2 x for 10 mins each.
15. Use Permount medium and coverslip, let it dry in the dark.