

TH DAB staining

1. Wash a full set of sections (6-8 sections) in a well in PBS, 5 min \times 3 (if stored in cryo-protective solution).
2. Incubate sections in 1% H₂O₂ (1 ml 30% H₂O₂ in 29 ml TBST), 30 min at RT.
3. Wash sections in TBST 5 min \times 3.
4. Block sections in 5% NGS/TBST, 1 hour at RT.
5. Incubate sections in rabbit anti-TH (#SA-497), **1:3000** in TBST, 4C overnight.
6. Wash sections in TBST, 10 min \times 3.
7. Incubate sections in anti-rabbit biotinylated (#OS03B), **1:3000**, 1hour at RT.
8. Prepare ABC (Avidin-Biotin-Complex, Vectastain ABC Kit, # PK-6100): 1 drop A and 1 drop B in 2.5 ml TBST, at least 30 min before use.
9. Wash sections in TBST, 10 min \times 3.
10. Incubate sections in ABC, 30 min at RT.
11. Prepare DAB solution (Sigma FAST, DAB Peroxidase Substrate Tablet Set), 1 DAB and 1 Urea Hydrogen Peroxide tablet in 15 ml distilled water.
12. Wash sections in TBST, 10 min \times 3.
13. Incubate sections in 1 ml DAB, 1-2 min.
14. Wash sections in TBST, 5 min \times 3.
15. Mount the sections on positive charged plus slides.