**IHC staining**

1. Bake the slides at 65 degrees for half an hour.
2. De-paraffin steps
3. Incubate slides in xylene I for 10 minutes
4. Incubate slides in xylene II for 10 minutes
5. Incubate slides in xylene III for 10 minutes
6. Incubate slides in 100% ethanol I for 10 minutes
7. Incubate slides in 100% ethanol II for 10 minutes
8. Incubate slides in 95% ethanol for 5 minutes
9. Incubate slides in 85% ethanol for 5 minutes
10. Incubate slides in 70% ethanol for 5 minutes
11. Incubate cassette in PBS for 5 minutes (3X)
12. Incubate slides in Hydrogen peroxidase blocking solution (3% H2O2) for 10 minutes and rinse with PBS for 5 minutes (3X)
13. The slides are heat boiling by microwave in Citrate Antigen Retrieval solution for 15 minutes (vector laboratory H-3300).
14. Rinse with PBS for 5 minutes (3X)
15. The slides are incubated with blocking reagents to block unspecific staining (Biocare medical BS996M)
16. The primary antibody is diluted (recommended dilution) in Antibody Diluent (Biocare medical PD900), incubate the sections overnight at 40 C
17. Rinse with PBS for 5 minutes (3X)
18. The slides are incubated with antibody enhancer for 10 minutes (GBI labs D39-110)
19. Wash with PBS for 5 minutes (3X)
20. The slides are incubated with HRP for rabbit antibody for 10 minutes (GBI labs D39-110)
21. Rinse with PBS for 5 minutes (3X)
22. The slides are incubated with DAB chromogen and substrate solution (BD pharmingenTM 550880), monitor the color development under microscope
23. Rinse with tap water for 5 minutes
24. The slides are incubated with counterstain hematoxylin solution for about 30 seconds. (Vector lab H-3404-100)
25. Rinse with tap water for 5 minutes
26. Incubate slides in 70% ethanol for 2 minutes
27. Incubate slides in 85% ethanol for 5 minutes
28. Incubate slides in 95% ethanol for 5 minutes
29. Incubate slides in 100% ethanol I for 10 minutes
30. Incubate slides in 100% ethanol II for 10 minutes
31. Incubate slides in xylene I for 10 minutes
32. Incubate slides in xylene II for 10 minutes
33. Incubate slides in xylene III for 10 minutes
34. Mounting the slides with cover glasses, air drying.