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NB100-105 Protocol

Immunohistochemistry Procedure (NB100-105)

Immunohistochemistry Procedure
1. If not previously done, bake sections at 60C for 30 minutes.
2. Hydrate sections through the following series.
A. 3 X 5 minutes xylenes
B. 3 X 5 minutes 100% Etoh
C. 2 minutes 95% Etoh
D. 2 minutes 70% Etoh
E. 1 minute 50% Etoh
F. 1 minute ddH2O
G. 1 minute TBS
3. If dry sections are needed, circle sample with wax pencil.
4. Antigen unmasking was performed by microwaving in 0.1M sodium citrate (pH 6.0) for 2 X 5 minutes at power level 7. Cool for 15 minutes.
5. Rinse slides with TBS.
6. Quench slides in 0.3% hydrogen peroxide in MeOH (0.5 ml 30% stock in 50 ml MeOH) for 25 minutes.
7. Wash 2 X 5 minutes with TBS.
8. Block sections with 10% serum (from the host species of the secondary antibody) in TBS for 30 minutes.
9. Incubate NB 100-105 with sections overnight at 4C at a 1:50 dilution in 10% goat serum.
10. The following day, allow sections to sit at RT for 30 minutes.
11. Wash sections 3 X 5 minutes in TBS, followed by incubation with the secondary antibody diluted 1:100 in 10% goat serum for 30 minutes at RT.
12. After 15 minutes of incubation, make up ABC solution and allow to sit for 30 minutes.
13. Wash slides 3 X 5 minutes in TBS.
14. Block with ABC solution in TBS.
15. Make DAB solution.
16. Incubate with fresh DAB solution until signal develops, then place in ddH2O. Dehydrate sections through graded alcohols to xylenes (reverse hydration steps) and coverslip with permount.