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IMMUNOHISTOCHEMISTRY-PARAFFIN PROTOCOL

A. Deparaffinization / hydrate

1. Incubate slide at 60℃ for 60 minutes.
2. Deparaffinize in xylene for 10 minutes and repeat one more times.
3. Hydrate in 100% alcohol for 5 minutes, in 95% alcohol for 5 minutes, in 85% alcohol for 5 minutes, in 75% alcohol for 5 minutes.
4. Dip into distill Water for 5 minutes.
5. Dip into TBS (50 mM Tris, 100 mM NaCl, pH 7.6), leave for 5 minutes, and repeat two times.

B. Antigen Retrieval

1. Add the appropriate antigen retrieval buffer (10 mM citrate buffer (pH6.0)) to the pressure cooker. Place the pressure cooker on the hotplate and turn it on full power. Do not secure the lid of the pressure cooker at this point, simply rest it on top. While waiting for the pressure cooker to come to a boil.
2. Once boiling, transfer the slides from the TBS to the pressure cooker. Secure the pressure cooker lid.
3. As soon as the cooker has reached full pressure, time 3 minutes.
4. When 3 minutes has elapsed, turn off the hotplate and place the pressure cooker in an empty sink.
5. Activate the pressure release valve and run cold water over the cooker. Once de-pressurized, open the lid and run cold water into the cooker.
6. Dip the slide in TBS for 5 minutes and repeat two times.
7. Immerse slides in 3% H2O2 (in fresh methanol) for 15 minutes at room temperature.
8. Wash with distilled water two times, 5 minutes each time.
9. Wash with TBS (pH 7.6) two times, 5 minutes each time.

C. Staining with Primary Antibody

1. Dilute primary antibody with 3% BSA in TBS. Cover the tissue section on the slide with diluted primary antibody (use 50 – 150μl for each slide).
2. Incubate at 37℃ for 30 minutes or at room temperature for 60 minutes (The optimal incubation time, incubation temperature, and antibody dilution should be determined by the individual laboratory).
3. Wash with TBS two times, 5 minutes each time.

D. Staining with Secondary Antibody

1. Incubate with 100-200 ul Polymer Enhancer. Incubate 30 minutes at 37℃.
2. Wash with TBS for 3 times, 5 minutes each time.
3. Incubate with 100-200 ul Polymerized HRP and incubate 30 minutes at 37℃
4. Wash with TBS for 3 times, 5 minutes each time.
5. Add DAB solution and incubate 3-10 minutes (The reaction progress and the optimal time should be determined according to microscope).
6. Wash with distilled water for 2 times, 5 minutes each time.
7. Counterstain sections in hematoxylin if required, wash with distilled water. Immerse slides in 0.1% HCl-ethanol for 1-10 seconds, wash with distilled water.
8. Dehydrate through 95% ethanol for 1 minute, 100% ethanol for 2×3min, xylene for 2×3min, and coverslip with mounting medium.