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Immunohistochemistry

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We use this protocol and it's working

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Abstract

Immunohistochemistry protocol



- 1 Wash sections 3 x 5 min with Tris buffered solution (TB).
- 2 **Antigen retrieval:** incubate sections 30 min in citrate buffer at 37°C.
- 3 Wash sections 3 x 5 min with TB.
- 4 Wash sections 10 min with Tris buffered-saline solution (TBS).
- 5 **Endogenous peroxidase blocking:** incubate sections in a solution consisting of 3% H₂O₂ + 10% methanol diluted in distilled water for 20 min.
- 6 Wash sections 3 x 15 min with TBS.
- 7 **Incubation in blocking solution:** Incubate sections in a solution consisting of 10% of normal serum (from the same species as the secondary antibody) + 0.3% of Triton X-100 diluted in TBS at RT for 3 hours.
- 8 **Primary antibody incubation:** incubate sections 64hr at 4°C with primary antibody diluted in blocking buffer. Negative controls are incubated with blocking buffer alone.
- 9 Wash sections 4 x 15 min with TBS.
- 10 **Secondary antibody incubation:** incubate sections 2hr at RT with biotinylated secondary antibody diluted in blocking buffer.
- 11 Wash sections 4 x 15 min with TBS.
- 12 **ABC kit incubation:** incubate sections in a solution with avidin-biotin horseradish peroxidase conjugate (ABC) for 45 min at RT.
- 13 Wash sections 2 x 15 min with TBS.



- 14 Wash sections 1 x 15 min with TB.
- 15 Signal was visualized incubating sections in a solution consisting of DAB 0.05% + H₂O₂ 0.003% diluted in TB during 5-10 min under microscope control.
- 16 Wash sections with distilled water and counterstain with hematoxylin for 3 min.
- 17 Wash section with running water for 2 min.
- 18 Dehydrate sections by immersion in ascending ethanol (5 min each) followed by clearing in xylene for 2 x 10 minutes.
- 19 Mount the cover slides with DPX.