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Protocol status: Working

Copy of Immunohistochemistry

PLOS One

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Wenxue Wu

MATERIALS

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- ✕ 0.1% Triton X-100-containing 1XPBS solution/5% normal goat serum **Contribute by users**
- ✕ Sodium phosphate dibasic **Sigma Aldrich Catalog #7558-79-4**
- ✕ Tween-20 **Sigma-aldrich Catalog #P-7949**
- ✕ Sensitive DAB Stain Kit **Bio Basic Inc. Catalog #PW023.SIZE.5Preps**
- ✕ EDTA **Invitrogen - Thermo Fisher Catalog #AM9261**
- ✕ Antibody Diluent OP Quanto **Thermo Fisher Scientific Catalog #TA-125-AD**
- ✕ Tris Buffered Saline & Tween 20 (20x) **Thermo Fisher Scientific Catalog #T999-TT**

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- 1 Deparaffinize/hydrate sections: a.Incubate sections in three washes of xylene for 5 minutes each.b.Incubate sections in two washes of 100% ethanol for 10 minutes each.c.Incubate sections in two washes of 95% ethanol for 10 minutes each.
- 2 Wash sections twice in dH2O for 5 minutes each.
- 3 Incubate sections in 3% hydrogen peroxide for 10 minutes.
- 4 Wash sections in wash buffer for 5 minutes.
- 5 Block each section with 100-400 µl blocking solution for 1 hour at room temperature.
- 6 Remove blocking solution and add 100-400 µl primary antibody diluted in recommended antibody diluent to each section. Incubate overnight at 4°C.
- 7 Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
- 8 Add 100-400 µl biotinylated secondary antibody, diluted in TBST per manufacturer's

recommendation, to each section. Incubate 30 minutes at room temperature.

- 9 If using ABC avidin/biotin method, prepare ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
- 10 Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
- 11 Add 100-400 μ l ABC reagent to each section and incubate for 30 minutes at room temperature.
- 12 Remove ABC reagent and wash sections three times in wash buffer for 5 minutes each.
- 13 Add 100-400 μ l DAB or suitable substrate to each section and monitor staining closely.
- 14 As soon as the sections develop, immerse slides in dH₂O.
- 15 If desired, counterstain sections in hematoxylin per manufacturer's instructions.
- 16 Wash sections in dH₂O two times for 5 minutes each.

- 17** Dehydrate sections: a.Incubate sections in 95% ethanol two times for 10 seconds each.b.Repeat in 100% ethanol, incubating sections two times for 10 seconds each.c.Repeat in xylene, incubating sections two times for 10 seconds each.
- 18** Mount coverslips.