Immunohistochemistry for Paraffin-Embedded Tissues

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Materials & Reagents:

- Xylene
- 75%, 90%, 100% ethanol
- 55°C incubator
- 10 mM sodium-citrate buffer (pH 6)
- Thermo Shandon Sequenza slide rack and coverplates (e.g. Thermo 739910)
- Thermo Scientific Shandon Coverplates (Fisher 72-110-017)
- Sequenza Slide Rack (Ted Pella 36105)
- Dulbecco's PBS
- Ca-Mg-free Dulbecco's PBS
- DAKO Dual Endogenous Enzyme Blocker (#S2003)
- Triton X-100
- Siglec-8-COMP (20 μg/ml); Siglec-9-COMP (15 μg/ml)
- Mouse anti-His IgG (2 μg/ml)
- AP-conjugated goat anti-mouse IgG (2μg/ml)
- Vector Red Substrate Kit (SK-5100)
- Vector Hematoxylin QS
- Mounting medium (Krystalon, HARLECO 64969-95)

Method:

1. Paraffin tissue sections on slides are stored at ambient temperature (RT)
2. Heat dry the sections at 55°C for 1-2 hours
3. Deparaffinize slides by sequential immersion into separate individual containers of:
    a. Xylene (5 min)
    b. Xylene (5 min)
    c. Xylene (5 min)
    d. 100% ethanol (3min)
    e. 95% ethanol (3min)
    f. 70% ethanol (3min)
4. Place the slides under running water for 5 min.
5. Retrieval of antigenicity: Place sections in 10 mM sodium citrate buffer pH 6 in a microwave on high until small bubble appear (1-2 min, sub-boiling). Repeat 3 times with 5-min intervals between heatings. Allow the slides to cool to RT (usually ≥20 min).
6. Wash the slides with PBS, cover each with a Shandon Coverplate, then insert into the Sequenza rack. Make sure there is water in the rack before inserting slides. For subsequent steps add solutions to the slides in the Sequenza rack.

7. Wash with 500 µl Ca-Mg-free PBS containing 0.1% Triton X-100.
8. Block with 200 µl 1% BSA in Ca-Mg-free PBS containing 0.1% Triton X-100 for 30 min.
9. Add 2 drops DAKO Dual Endogenous Enzyme Blocker for 10 min at RT.
10. Add 200 µl of Siglec-8-COMP (20 µg/ml) or Siglec-9-COMP (15 µg/ml) for 1 h.
11. Wash with 500 µl Ca-Mg-free PBS containing 0.1% Triton X-100 three times.
12. Add 200 µl mouse anti-His IgG (2µg/ml)
13. Wash with 500 µl Ca-Mg-free PBS containing 0.1% Triton X-100 three times.
14. Add 200 µl goat anti-mouse IgG- AP (2 µg/ml)
15. Wash with 500 µl Ca-Mg-free PBS containing 0.1% Triton X-100 three times.
16. Remove from Sequenza rack and develop by immersion in Vector Red Substrate for 20 min.
17. Wash with water
18. Counterstain with Hematoxylin QS (Vector H3404)
19. Dehydrate sections by sequential immersion into separate individual containers of:
    a. 70% ethanol (3min)
    b. 95% ethanol (3min)
    c. 100% ethanol (3min)
    d. Xylene (5 min)
    e. Xylene (5 min)
    f. Xylene (5 min)
20. Allow xylene to evaporate and coverslip with mounting medium
21. Allow to harden for 1 h, then collect microscopic images

NOTE:

- Use different ethanol solutions for Step 19 than you did for Step 3 (xylene solutions can be the same).