Detection of lectin ligands using formalin-fixed paraffin-embedded (FFPE) tissue sections

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Materials & Reagents:

- Xylene
- 75%, 90%, 100% ethanol
- 55°C incubator
- 10 mM sodium citrate buffer (pH 6)
- Ca-Mg-free Dulbecco's PBS
- DAKO Dual Endogenous Enzyme Blocker (#S2003)
- Human Fc Receptor Blocker (INNOVEX NB309)
- Triton X-100
- Siglec-8 Fc; Siglec-9 Fc; Siglec-F Fc
- AP-conjugated goat anti-human Fc (Jackson109055008, 0.3 mg/ml)
- Vector Red Substrate Kit (SK5100)
- Vector Hematoxylin QS (H3404)
- Mounting medium (Krystalon, HARLECO 64969-95)
- Coplin jars
- Glass tubs for washing

Method:

1. Incubate the slides at 55°C for 1 h to dry and to melt the paraffin.
2. Deparaffinize slides by sequential immersion into separate individual Coplin jars of:
    a. Xylene (5 min) Agitate occasionally. If sections are very small, 3 min is enough.
    b. Xylene (5 min)
    c. Xylene (5 min)
    d. 100% ethanol (3min)
    e. 95% ethanol (3min)
    f. 70% ethanol (3min)
3. Rinse the slides in water. Place the slides in water for 10 min to rehydrate. Heat up solution for next step while waiting.
4. "Antigen Retrieval": Place sections in near boiling 10 mM sodium citrate buffer pH 6 for 5 min. Then heat in a microwave on high until small bubbles appear (1-2 min, sub-boiling). Repeat 2 times with 5-min intervals between heatings. Allow the slides to cool to ambient temperature.
5. Wash twice for 5 mins in PBS in slide washing racks on the shaker. Dry the slides and draw a barrier around the tissue with a hydrophobic pen.
6. To block, add ~200 µl of 30 mg/ml BSA in Ca-Mg-free PBS containing 0.1% Triton X-100 (PBSTr) directly onto the slides. Incubate for 30 min.
7. Remove the blocking buffer and add 2 drops DAKO Dual Endogenous Enzyme Blocker for 10

min at RT. Then wash twice for 5 min in 0.1%PBSTr on the shaker.

8. Add 2 drops of Fc blocker directly on the slides, incubate for 30 min at room temperature. Then wash twice for 5 min in 0.1% PBSTr.

- In the meantime, precomplex Siglec-Fc and secondary antibody in 1% BSA/PBSTr: Add Siglec-Fc (final concentration 15 µg/ml) and AP conjugated goat anti-human Fc in a 2:1 molar ratio in 1%BSA/PBSTr. Incubate at 4°C for 20 min.

9. Add 200 µl of precomplexed Siglec-Fc solution and incubate overnight at 4C.
10. After the slides are incubated with Siglec-Fc, wash in PBSTr three times 10 min each.
11. If using AP secondary antibodies: wash one time with 100mM Tris-Cl, 0.1% Tween-20, pH 8.3 for 5 min. (Vector Red developing buffer)
12. Develop by adding Vector Red Substrate directly onto the slides. Incubate for 2 min. (Short incubation time may be needed to prevent high background). Rinse in a slide rack under running tap water till water is colorless.
13. Add 2 drops of Hematoxylin QS (Vector H3404) for counterstaining for 10 to 15 seconds. Wash with water till water is colorless.
14. Dehydrate sections by sequential immersion into separate individual containers of:
    a. 70% ethanol (fresh ethanol! Don't use the left over from Step 2)
    b. 95% ethanol
    c. 100% ethanol
    d. Xylene (May use the same xylene from Step 2)
    e. Xylene
    f. Xylene
15. Allow xylene to evaporate and coverslip with mounting medium
16. Allow to harden for 1 h, then collect microscopic images