Lectins on paraffin
Date: Lectin histochemistry protocol

Materials:
1. Paraffin sections of organs: Mouse spleen is a good control
2. Wash buffer : 0.05 M Tris HCl/ 150 mM NaCl pH 8.0 / 0.1% Tween 20
3. Diluting buffer : wash buffer with 1% BSA: 500 mg /50 ml of wash buffer.
4. Add $CaCl_2$ (10mM) and $MnCl_2$ (10 mM) to diluting buffer just before assay

Procedure:

1. De-paraffinize and rehydrate sections on slides: xylene-3 changes, 100% alcohol-3 changes; 95% alcohol – 3 changes, 70% alcohol – 3 changes, TBST washes x3
2. Block endogenous peroxidases: 0.3% H^2O^2 for 30 minutes at room temperature followed by 3 buffer washes, if using HRP labeled secondary
3. Block endogenous biotin:
   a) Overlay with 0.1% avidin/PBS for 15 minutes; TBST washes x3
   b) Overlay with 0.01% biotin/PBS for 15 minutes; TBST washes x 3
4. Primary reagent incubation:
   overlay with negative control or with diluted lectin:
   a) diluting buffer control slide
   b) biotinylated SNA 1:500 (Vector Labs B1305, Elderberry bark lectin)
   c) biotinylated PNA 1:1000 (Vector labs B1075, Peanut Agglutinin)
   d) biotinylated ECA 1: 2500 (Vector labs B1145, Erythrina Cristagalli)
   e) Biotinylated GSL1 1:2000 (Vector Labs B1105, Griffonia Simplicifolia lectin)
   f) biotinylated MAL 2 1:500 (Vector labs B1265, Maackia Amurensis lectin 2)
   g) biotinylated UEA 1:500 (Vector labs B1065, Ulex Europaeus agglutinin I)
   h) biotinylated TL 1: 1000 (Vector labs B1175, Lycopersicon Esculentum-tomato lectin)

   incubate 30 minutes at room temperature; or one hour or overnight in refrigerator,
   wash in 3 changes of wash buffer

6. Secondary : Overlay with labeled secondary: incubate for 30 minutes at room temperature;
   wash in 3 changes of washing buffer.
   a. Alkaline phosphatase labeled streptavidin (Jackson labs 016-050-084) 1:100 in diluting buffer
   OR with
   b. HRP-streptavidin (Jackson Immunoresearch) 1:500 in diluting buffer
7. Make fresh substrate; overlay with substrate for 3-5 minutes (Vector Blue in 0.1M Tris /levimasole-- Vector labs SK-5300 Alkaline phosphatase substrate III)
   or with AEC if using HRP labeled secondary. wash with 3 changes of wash buffer
8. Nuclear counterstain in nuclear fast red for 30 minutes (Vector labs ----)
   OR Meyer's hematoxylin for 3 minutes; wash with 3 changes of wash buffer
9. coverslip with aquamount

Reagents:
1. Bovine Serum Albumin: Sigma: A4503-50G
2. Hydrogen Peroxide to block endogenous peroxidases: Fisher: H325-100
3. Avidin Biotin Blocking agent Vector labs SP2001
4. Alkaline phosphatase labeled streptavidin (Jackson labs 016-050-084) 1:100
5. Vector Blue Alkaline phosphatase substrate : Vector labs SK-5300 : made fresh in 0.1M Tris /levimasole--
6. Horse radish Peroxidase labeled streptavidin (Jackson labs 016-030-084) 1:500
7. AEC Peroxidase substrate kit Vector labs SK 4200

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8. Nuclear counterstain if using AEC substrate for HRP: Mayers' Hematoxylin Sigma Cat No MH532-IL
9. Nuclear counterstain if using Alkaline Phosphastase Vector Blue Substrate: Nuclear Fast Red (Vector labs ----
10. Aqueous mounting with coverslips:

---SNA: Sambucus nigra agglutinin--α *2-6 linked sialic acids*

--- ECA: Erythrina crystagalli agglutinin-- *Galβ 1-4 GlcNAc*

---PNA: Peanut agglutinin-- *Gal β1-3 GalNAc*

---MAH: MaackiaAmurensisHemagglutinin--α*2-3 linked sialic acids*

---GSL1: Griffonia Simplicifolia Lectin 1---- α *galactose*

---TL: Tomato lectin, Lycopersicon Esculentum----*poly lactosamines*

---L-PHA: Phaseolus Vulgaris Leucoagglutinin----*complex structures*

---UEA: Ulex europaeus agglutinin----α *linked fucose residues*